REMARKS

A check for the fee for a three month extension of time accompanies this response. Any fees that may be due in connection with filing this paper or with this application during its entire pendency may be charged to Deposit Account No. **06-1050**. If a Petition for extension of time is required, this paper is to be considered such Petition, and any fee charged to Deposit Account No. **06-1050**. A change of address for the undersigned accompanies this response.

Claims 50-52 are pending in this application. A DECLARATION under 37 C.F.R. §1.132 of Dr. Steven Fabijanski accompanies this response.

THE REJECTION OF CLAIMS 50-52 UNDER 35 U.S.C. §112, SECOND PARAGRAPH

Claims 50-52 are rejected under 35 U.S.C. §112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter that Applicant regards as the invention. Specifically, the Office Action alleges that the term "satellite artificial chromosome" is unclear. The Office Action further alleges that the instant application, while reciting the "properties" of a satellite artificial chromosome, does not teach the essential elements of a satellite artificial chromosome, nor the metes and bounds of a satellite artificial chromosome. It is further alleged that there is no teaching of the components of a plant satellite artificial chromosome, nor of introducing a plant satellite artificial chromosome into a plant cell. This rejection is respectfully traversed.

RELEVANT LAW

Claims are not read in a vacuum but instead are considered in light of the specification and the general understanding of the skilled artisan. Rosemount Inc. v. Beckman Instruments, Inc., 727 F.2d 1540, 1547, 221 USPQ 1, 7 (Fed. Cir. 1984), Caterpillar Tractor Co. v. Berco, S.P.A., 714 F.2d 1110, 1116, 219 USPQ 185, 188 (Fed. Cir. 1983). When one skilled in the art would understand

all of the language in the claims when read in light of the specification, a claim is not indefinite.

There are no requirements for terms to be defined in the claims when one of skill in the art can readily determine the meaning of the term based on the description and definitions provided in the specification. In this respect, an applicant is entitled to be its own lexicographer [see, *e.g.*, MPEP 2111.01 "Applicant may be his or her own lexicographer as long as the meaning assigned to the term is not repugnant to the term's well known usage and utilize terms within the claims that are clear from a reading of the specification. In re Hill, 73 USPQ 482 (CCPA 1947)."]. When applicant has provided definitions in the specification, the claims are interpreted in light of such definition.

35 U.S.C. § 112, second paragraph, requires only reasonable precision in delineating the bounds of the claimed invention. Claim language is satisfactory if it reasonably apprises those of skill in the art of the bounds of the claimed invention and is as precise as the subject matter permits. Shatterproof Glass Corp. v. Libby-Owens Ford Col., 758 F.2d 613, 624, 225 USPQ 634, 641 (Fed. Cir.), cert. dismissed, 106 S.Ct. 340 (1985).

The amount of detail required to be included in the claims depends on the particular subject matter and the prior art and is not to be viewed in the abstract, but in conjunction with whether the specification is in compliance with the first paragraph of 35 U.S.C. § 112. If the claims, read in light of the specification, reasonably apprise those skilled in the art of the utilization and scope of the invention, and if the language is as precise as the subject matter permits, the courts can demand no more:

> [i]t is not necessary that a claim recite each and every element needed for the practical utilization of the claimed subject matter (Bendix Corp. v. United States, 600 F.2d 1364, 1369, 220 Ct. Cl. 507, 514, 204 USPQ 617, 621 (1979); See, also, Carl Zeiss Stiftung v. Renishaw plc, 20 USPQ2d 1094, 1101).

ANALYSIS

Applicant respectfully submits that the term "satellite artificial chromosome" is definite. The elements of a satellite artificial chromosome are clearly defined in the specification so that the metes and bounds of the term "satellite artificial chromosome", in light of the specification and as understood by those of skill in the art, are clear.

First, it is noted that Applicant has discovered and provides something so new that no name existed to describe the chromosomes. Before someone had built the first automobile or telephone, the terms "automobile" or "telephone" would not have been clear. Just as the inventor of each of these items had to name them, Applicant coined its own name for its new vectors, and called them "satellite artificial chromosomes."

Further, it is respectfully submitted that the term "satellite artificial chromosome" is discussed at great length throughout the specification. The term " satellite" refers to the repeating units of short satellite DNA that primarily make up the satellite artificial chromosome. As is known to one of skill in the art and as defined herein (*see, e.g.*, page 17, lines 30-31), satellite DNA is highly repetitive, non-coding DNA found primarily in centromeric regions of chromosomal DNA.

A satellite artificial chromosome is defined in the specification as a chromosome that contains more heterochromatin than euchromatin and can include portions of heterologous DNA" (*see, e.g.*, page 18, lines 23-25; page 19, lines 3-5; page 19, lines 3-8). Page 17, lines 28-30, of the specification defines heterochromatin as chromatin that is unusually condensed and thought to be transcriptionally inactive, and page 21, lines 28-30, defines heterologous DNA as DNA that does not occur naturally as part of the genome. Page 22,

lines 2-12 of the specification provides examples of heterologous DNA that can form part of the satellite artificial chromosome.

Satellite artificial chromosomes can be identified, for example, in that they can be generated *de novo* in eukaryotic cells and result from large-scale amplification of specific chromosomal regions (*see, e.g.*, Figure 2 and its description in the specification beginning at page 15). The repeat structure of satellite artificial chromosomes is described in the specification, *e.g.*, at page 8, lines 18-30:

> Also provided are SATACs of various sizes that are formed by repeated culturing under selective conditions and subcloning of cells that contain chromosomes produced from the formerly dicentric chromosomes. **The exemplified SATACs are based on repeating DNA units** that are about 15 Mb [two ~7.5 Mb blocks]. The repeating DNA unit of SATACs formed from other species and other chromosomes may vary, but typically would be on the order of about 7 to about 20 Mb. **The repeating DNA units are referred to herein as megareplicons, which in the exemplified SATACs contain tandem blocks of satellite DNA flanked by non-satellite DNA, including heterologous DNA and non-satellite DNA.** Amplification produces an array of chromosome segments [each called an amplicon] that contain two inverted megareplicons bordered by heterologous ["foreign"] DNA.

Thus, the specification clearly defines a satellite artificial chromosome as one that contains a tandem array of identical chromosome segments (each called an amplicon) that contain two inverted megareplicons bordered by heterologous DNA. Satellite artificial chromosomes are based on these repeating units (megareplicons), clearly defined as containing tandem blocks of satellite DNA flanked by non-satellite DNA, and can be produced to include heterologous DNA that is expressed.

These heterochromatic chromosomes can be distinguished from, for example, minichromosomes formed by amplification of euchromatin, which is defined in the specification as chromatin that stains diffusely and that typically contains genes (*see, e.g.*, page 17, lines 27-28). Further, satellite artificial

chromosomes are distinguished in that they "are nearly fully heterochromatic," so that without insertion of heterologous or foreign DNA, the chromosomes contain no genetic information or contain only non-protein-encoding gene sequences, such as rDNA sequences.

The specification further identifies and characterizes the satellite artificial chromosomes and each of the intermediates generated in the process of *de novo* satellite artificial chromosome formation. Extensive analyses using methods including Southern hybridization, long-range mapping of restriction endonuclease sites, indirect immunofluorescence with anti-centromere antibodies provide definition of the artificial chromosomes at the level of the basic structural and functional elements that comprise these chromosomes, including the characteristic repeated units of satellite and foreign DNA. Many of these features are depicted schematically in Figures 1-3 of the application. In view of the extensive description and in-depth treatment in the application, it is respectfully submitted that the metes and bounds of the term, "satellite artificial chromosome" is definite and understood without any further clarification in the claims.

Further, contrary to the Examiner's assertion, the metes and bounds of <u>any</u> satellite artificial chromosome, including a plant satellite artificial chromosome, are clear in light of the characterization in the specification. For example, at page 16, lines 18-25, of the instant application, the specification teaches that plant artificial chromosomes have the same structural elements as described for mammalian artificial chromosomes, with the exception of having a plant centromere instead of a mammalian centromere. The specification describes these structural elements, which include centromeres, telomeres, an origin of replication and heterochromatin (*see*, for example, page 7, lines 9-18 and page 10, lines 2-10).

The Office Action alleges that the specification provides no teaching of how to introduce a plant satellite artificial chromosome into a plant cell. It

appears that the Examiner is alleging that the disclosure is not enabling for the step of introducing a SATAC into a protoplast, and this is addressed below in response to the rejection on the grounds of inadequate scope of enablement. As discussed below, the specification describes in great detail the introduction of DNA, including chromosomes, into a variety of cells, including protoplasts; therefore, the step of introducing a SATAC into a protoplast as recited in the instant claims is clear in light of the detailed description in the specification.

The Office Action alleges that the application recites "properties" of a satellite artificial chromosome without teaching what its "essential elements" are. To the contrary, as discussed above, the satellite artificial chromosomes are characterized in the specification in terms of extensive structural detail of their elements. As also discussed, Applicant's discovery is of a pioneering nature, containing and defining several terms that have never before been described in the prior art; accordingly, the details provided by Applicant should not be expected to refer to prior known terms, when there have been no such terms.

Each of the terms pointed to by the Examiner (page 3, subheading no. 6) as allegedly unclear are in fact clearly defined. These terms include:

1) "satellite DNA:" As explained above, this is a term of art used to refer to highly repetitive DNA (see for example, page 17, lines 30-31).

2) "fully functional stable chromosome:" the elements of a functional, stable, artificial chromosome are provided, *e.g.*, at page 10, lines 7-9 and include a centromere, two telomeres, at least one origin of replication and filler heterochromatin, *e.g.*, satellite DNA.

3) "provides an extra genomic locus for targeted integration of DNA:" the specification provides examples of such loci for targeted integration of DNA into a satellite artificial chromosome, including pericentric heterochromatin and rDNA in centromeric regions of chromosomes containing rDNA sequences (*see, e.g.*, page 33, line 30 to page 34, line 2).

4) "repeating units of short satellite DNA:" As discussed in detail above, the specification (*see, e.g.*, page 8, lines 18-30) clearly defines a satellite artificial chromosome as one that is based on repeating units called megareplicons, which in turn are clearly defined as containing tandem blocks of satellite DNA flanked by non-satellite DNA.

5) "nearly fully heterochromatic:" The instant application defines "nearly fully heterochromatic" on page 7 as "so that without insertion of heterologous or foreign DNA, the chromosomes preferably contain no genetic information or contain only non-protein-encoding gene sequences such as rDNA" (lines 11-14). Further, as discussed above, heterochromatin is defined as unusually condensed chromatin that is typically transcriptionally inactive (*see, e.g.*, page 17, lines 28-30).

Therefore, it is respectfully submitted that in light of the detailed structural characterizations and definitions provided in the specification, the terms alleged to be recitations of satellite artificial chromosomes, are in fact elements of satellite artificial chromosomes. Further, the definition of each of these elements renders the metes and bounds of the term "satellite artificial chromosomes" definite without further clarification.

In addition to defining and extensively characterizing satellite artificial chromosomes in the instant application so that the metes and bounds of a satellite artificial chromosome are clear, the Examiner's attention is directed to U.S. Patent Nos. 6,077,697 and 6,025,155, granted to Hadlaczky *et al.* This application is a continuation-in-part of each of the applications upon which the US patents are based. Each of these applications has issued claims to satellite artificial chromosomes. For example, in U.S. Patent No. 6,077,697, Claim 8 recites:

8. An isolated substantially pure satellite artificial chromosome.

Because an issued patent is presumed valid (35 U.S.C.§ 282), and the term "satellite artificial chromosome" is recited in the issued claims, the term "satellite artificial chromosome" should be considered presumptively definite.

Accordingly, it is respectfully submitted that Claims 50-52 of the instant application are not indefinite.

THE REJECTION OF CLAIMS 50-52 UNDER 35 U.S.C. §112, FIRST PARAGRAPH

The written description rejection

Claims 50-52 are rejected under 35 U.S.C. § 112, first paragraph, because it is alleged that the specification does not describe the subject matter in such a way as to convey to one skilled in the relevant art that the inventor(s) had possession of the claimed subject matter at the time the application was filed. In particular, it is alleged that the identifying characteristics set forth in the specification for a satellite artificial chromosome or a plant artificial chromosome are insufficient to demonstrate possession of satellite artificial chromosomes and plant satellite artificial chromosomes by the Applicant. Further, it is alleged that insufficient relevant identifying characteristics are set forth to "predictably" determine the structure and function of a satellite artificial chromosome in order to use the satellite artificial chromosome as the starting material for producing a cell containing a satellite artificial chromosome or a plant satellite artificial chromosome and then identifying the cell containing the satellite artificial chromosome or plant satellite artificial chromosome.

This rejection is respectfully traversed.

Relevant Law

The purpose behind the written description requirement is to ensure that the patent applicant had possession of the claimed subject mater at the time of filing of the application In re Wertheim, 541 F.2d 257, 262, 191 USPQ 90, 96 (CCPA 1976). The manner in which the specification meets the requirement is not material; it may be met by either an express or an implicit disclosure.

35 U.S.C. §112 requires a written description of the invention. This requirement is distinct from and not coterminous with the enablement requirement:

> The purpose of the 'written description' requirement is broader than to merely explain how to 'make and use'; the applicant must also convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention. The invention is, for purposes of the 'written description' inquiry, whatever is now claimed." Vas-Cath, Inc. v. Mahurkar, 935 F.2d at 1563-64, 19 USPQ2d at 1117 (emphasis in original).

The issue with respect to 35 U.S.C. §112, first paragraph, adequate written description has been stated as:

> [d]oes the specification convey clearly to those skilled in the art, to whom it is addressed, in any way, the information that appellants invented that specific compound [claimed embodiment] Vas-Cath, Inc. v. Mahurkar, at 1115, quoting In re Ruschig, 390 F.2d 1990, at 995-996, 154 USPQ 118 at 123 (CCPA 1967).

A specification must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention, *i.e.*, whatever is now claimed. Vas-Cath, Inc. v. Mahurkar, 935 F.2d 1555, 1563-64, 19 USPQ.2d 1111, 1117 (Fed. Cir. 1991). A written description requirement issue generally involves the question of whether the subject matter of a claim is supported by or conforms to the disclosure of an application as filed. The test for sufficiency of support in a patent application is whether the disclosure of the application relied upon "reasonably conveys to the artisan that the inventor had possession at that time of the later claimed subject matter." Ralston Purina Co. v. Far-Mar-Co., Inc., 772 F.2d 1570, 1575, 227 USPQ 177, 179 (Fed. Cir. 1985) (quoting In re Kaslow, 707 F.2d 1366, 1375, 217 USPQ 1089, 1096 (Fed. Cir. 1983)) (see also, MPEP 2163.02).

An objective standard for determining compliance with the written description requirement is "does the description clearly allow persons of skill in

the art to recognize that he or she invented what is claimed." In re Gosteli, 872 F.2d 1008, 1012, 10 USPQ.2d 1614, 1618 (Fed. Cir.1989).

The Examiner has the initial burden of presenting evidence or reasons why persons skilled in the art would not recognize in an applicant's disclosure a description of the invention defined by the claims. In re Wertheim, 541 F.2d 257, 265, 191 USPQ 90, 98 (CCPA 1976); *See also* Ex parte Sorenson, 3 USPQ.2d 1462, 1463 (Bd. Pat.App. & Inter. 1987). By disclosing in a patent application a device that inherently performs a function or has a property, operates according to a theory or has an advantage, a patent application necessarily discloses that function, theory or advantage, even though it says nothing explicit concerning it. The application may later be amended to recite the function, theory or advantage without introducing prohibited new matter. In re Reynolds, 443 F.2d 384, 170 USPQ 94 (CCPA 1971); and In re Smythe, 480 F. 2d 1376, 178 USPQ 279 (CCPA 1973).

Furthermore, the subject matter of the claims need not be described literally (*i.e.*, using the same terms or *inhaec verba*) in order for the disclosure to satisfy the description requirement. If a claim is amended to include subject matter, limitations, or terminology not present in the application as filed, involving a departure from, addition to, or deletion from the disclosure of the application as filed, the examiner should conclude that the claimed subject matter is not described in that application. This conclusion will result in the rejection of the claims affected under 35 U.S.C.112, first paragraph - description requirement, or denial of the benefit of the filing date of a previously filed application, as appropriate.

Analysis

1) The Office Action alleges that satellite artificial chromosomes and plant satellite artificial chromosomes are not disclosed with sufficient identifying characteristics to be considered to be possessed by Applicant.

The claims of the instant application are drawn to methods for producing a transgenic plant by introducing a SATAC into a protoplast. As discussed above with respect to the rejection on grounds of indefiniteness, the specification provides detailed definitions and structural characterizations of a satellite artificial chromosome (SATAC) and each of its elements so that it is clear that Applicant was in possession of a SATAC as of the filing date of the instant application and as of its earliest priority date.

The specification describes how plant artificial chromosomes differ from other artificial chromosomes, for example, at page 16, lines 18-25, of the instant application:

> [A] mammalian artificial chromosome [MAC] is a piece of DNA that can stably replicate and segregate alongside endogenous chromosomes. It has the capacity to accommodate and express heterologous genes inserted therein. It is referred to as a mammalian artificial chromosome because it includes an active mammalian centromere(s). **Plant artificial chromosomes**, insect artificial chromosomes and avian artificial chromosomes **refer to chromosomes that include plant** and insect **centromeres**, respectively. (emphasis added)

Thus, plant artificial chromosomes have the same structural elements as described for mammalian artificial chromosomes, with the exception of having a plant centromere instead of a mammalian centromere. The specification describes these structural elements, which include centromeres, telomeres, an origin of replication and heterochromatin (*see*, for example, page 7, lines 9-18 and page 10, lines 2-10).

For example, at page 43, the instant application begins a section entitled "Identification and isolation of the components of artificial chromosomes." Within this section are subheadings, centromeres (page 44, line 18), telomeres (page 46, line 1), megareplicator (page 46, line 12), filler heterochromatin (page 46, line 20) and selectable marker (page 47, line 8); the subsections each describe an element of SATACs in detail. Thus, the structural elements of

SATACs, including plant SATACs, are both identified and described in the instant application.

The specification describes the development of a satellite artificial chromosome containing sequences that can be of animal or plant origin and include centromere-related sequences. For example, the specification describes methods for generating species-specific satellite artificial chromosomes by adding a centromere from other species, including plants (see, *e.g.*, at page 12, lines 3-16). In the specification, *e.g.*, at page 11, line 30, through page 12, line 16, a method for cloning a centromere in a selected species (*e.g.*, plants) is described. These methods for cloning centromeres from a selected animal or plant include: (i) preparing a library of DNA fragments that contains the genome of the plant or animal; (ii) introducing each of the fragments into a mammalian satellite artificial chromosome that contains a selectable marker and a centromere from a species different from the selected plant or animal; (iii) introducing each of the satellite artificial chromosomes into a cell, which is grown under selective conditions; and (iv) selecting cells containing satellite artificial chromosomes. Satellite artificial chromosomes that are identified by the methods provides herein should contain a centromere encoded by the DNA from the library, be it plant DNA or mammalian DNA, and should contain the necessary elements for stable replication in the selected species. Thus, the specification provides methods where a satellite artificial chromosome is developed in one source cell, modified to contain sequences specific to the centromere region of a target cell and then transferred to the target cell type.

Methods for the modification of satellite artificial chromosomes are described in extensive detail in the specification (*e.g.*, at page 39, line 26, through page 40, line 21; and page 150, through page 157). The cited passage(s) describe the use of homologous recombination to insert new DNA fragments into a satellite artificial chromosome. Accordingly, methods to

generate and modify a satellite artificial chromosome to contain a plant centromere DNA sequence have been provided.

Further, it is not necessary to include in the specification that which those of skill in the art know. The specification is presumed to include all such knowledge. For example, as of the effective filing date, plant centromere sequences were known in the art, such as *Arabidopsis* rDNA and 180 bp repeat sequences and *Brassica campestris* 175 bp repeat sequences (*see*, for example, Murata *et al.* (1994) *Jpn. J. Genet.* 69: 361-70; Maluszynska *et al.* (1991) *Plant J. 1*:159-66; Genbank sequence X52320). Additionally, as provided in the previous Response filed July 16, 2003, available sequence information for plant centromeres, telomeres and autonomously replicating sequences (ARS) included for example, Jiang *et al. Proc Natl Acad Sci USA, 93*:14210-14213 (1996); Richards, E.J. "Plant Telomeres" in *Telomeres* Eds. C. Greider, and E.H. Blackburn, Cold Spring Harbor Laboratory Press, Cold Spring Harbor (1995); Rhodes *et al. Curr. Opin. Struct. Biol. 5(3):* 311-322 (1995); Zakian *Science 270(5242):* 1601-1607 (1995); Berlani *et al. Plant Mol. Biol. 11:* 161-162 (1988); Berlani *et al. Plant Mol. Biol. 11:* 173-182 (1988); and Eckdahl *et al. Plant Mol. Biol. 12:* 507-516 (1989)). Methods for the isolation of plant centromere DNA sequences were also available (*see*, *e.g.*, Jiang *et al. Proc Natl Acad Sci USA, 93*:14210-14213 (1996); Kaszas *et al. EMBO J. 15:* 5246-5255 (1996); Frary *et al. Mol. Genet. 250:* 295-340 (1996)).

Additionally, the instant application provides exemplary SATACs evidencing Applicant's possession of the claimed subject matter. For example, the specification describes the generation of cell lines such as G3D5 and H1D3, containing megachromosomes (exemplary SATACs). The cell lines have been deposited with the ECACC under accession nos. 96040928 and 96040929, respectively (page 74, lines 26-27). The specification also depicts the structures of SATACs schematically in Figures 2 and 3. In particular, a method

for formation of a megachromosome is shown in Figures 2D, 2E and 2F and an exemplary megachromosome structure is depicted in Figures 2F and 3.

The specification describes isolation and purification of SATACS from cells. For example, starting on page 41, the application describes isolation of SATACS using FACS sorting and affinity purification methods. These methods are exemplified in EXAMPLE 10, which details purification methods, including purity assessment and characteristics of the isolated SATACS, and demonstrates microcell fusion for transfer of SATACS,

The instant application also provides exemplary elements of SATACs. The specification provides exemplary telomeres such as the human telomere sequence in SEQ ID:3 (see Example 12 page 141, lines 23-25) and the construction of a 1 Kb synthetic telomere in Example 12 (pages 142-146). Exemplary centromeres are provided, for example, the centromeres of the megachromosomes in H1D3 and derivatives thereof, such as mM2C1 cells. The specification describes the megareplicator element as providing an origin of replication and sequences that facilitate amplification of the artificial chromosome (page 46, lines 13-16). Exemplary megareplicator sequences, such as rDNA sequences SEQ ID NOs: 16 and 18-24, are provided. Filler heterochromatin is exemplified, such as satellite DNA including an A/T-rich DNA sequence of mouse major satellite DNA and a G/C-rich hamster natural satellite DNA. Additional exemplary satellite DNA sequences provided include monotone, tandem repeats of highly A/T- or G/C-rich DNA units (see for example page 46, lines 21-31), and a 3.2-kb fragment of a Chinese hamster chromosome 2-specific satellite DNA (*see* page 71, lines 23-25 and Fatyol *et al.* (1994) *Nuc. Acids Res.* 22:3728-3736).

In addition, the instant application is a continuation-in-part of Patent No. 6,077,697, which contains issued claims directed to methods of producing and isolating SATACs as well as composition claims directed to isolated SATACs. Thus, it is respectfully submitted that the specification describes in exquisite

detail and provides SATACs, including plant SATACs, and their structural elements. Therefore, Applicant was clearly in possession of SATACs as of the filing date of the instant application and as of its earliest priority date.

2) The Office Action alleges that there are insufficient identifying characteristics to "predictably" determine the structure and function of a SATAC in order to use the SATAC as a starting material for producing a cell that contains a SATAC or a plant SATAC and then identifying the cell containing a SATAC or plant SATAC.

First, Applicant respectfully submits that the question of "predictably" determining the structure and function of a SATAC or using a SATAC as starting material for producing a cell containing a SATAC or plant SATAC applies to enablement and not written description. The issue of predictability is addressed in detail below, when discussing the enablement rejection.

Further, as discussed in detail above, SATACs are described and exemplary SATACs are provided, the elements of SATACs are described and exemplary elements are provided, schematic representations of exemplary SATACs are shown in the figures, and exemplary cell lines containing SATACs are described and also deposited with ECACC. Therefore, notwithstanding the impropriety of applying "predictability" to an assessment of written description, the specification does in fact describe SATACs and cells containing SATACs in extensive detail so that SATACs and cells containing SATACs can be reproducibly and "predictably" identified.

The primary purpose of the written description requirement is to demonstrate that the Applicant was in possession of the invention at the time of the filing date sought. Vas-Cath, Inc. v. Mahurkar, 935 F.2d 1555, 1563-64, 19 USPQ.2d 1111, 1117 (Fed. Cir. 1991). As addressed above, the instant application provides sufficient description of relevant, identifying characteristics of SATACs and plant SATACs to evidence Applicant's possession of the claimed subject matter at the time of filing.

The Office Action cites University of California v. Eli Lilly. 119 F.3d 1559, 43 USPQ 2d 1398 (Fed. Cir. 1997) for the premise that naming a material is not adequate written description. Unlike Eli Lilly, however, where the patent at issue claimed a composition, Id. at 1566, the instant application is directed to methods. Additionally, unlike Eli Lilly, where the cDNA was not described by structural features, Id. at 1567, the instant application describes in great detail the structural and functional features of SATACs, including plant SATACs. The specification describes in extensive detail the preparation, characterization and isolation of satellite artificial chromosomes and types thereof such as megachromosomes, and provides numerous examples of particular embodiments thereof and cells containing particular embodiments thereof.

Furthermore, the court stated in Eli Lilly, that a description of a genus may be achieved by providing structural features common to the members of the genus. Id. at 1569. This test for the written description requirement is reiterated in the MPEP § 2163, which states that an adequate written description for a claimed genus need only provide "relevant, identifying characteristics" of a representative number of species. The instant application clearly describes the relevant identifying characteristics of SATACs. As discussed above, SATACs are described by their structural elements, including centromeres, telomeres, an origin of replication and containing predominantly heterochromatin. Plant SATACs are further described as a species of SATAC containing a plant centromere (see for example, page 16, lines 23-25). Thus, Applicant has complied with the expectations set forth in the written description requirement by describing the relevant, identifying structural features of SATACs that are common to the genus. Therefore, Applicant has sufficiently described SATACs and plant SATACs such that they can be used in the claimed methods for introducing a SATAC into a cell.

The Applicant, in the instant specification, also describes the exact steps involved in generating satellite artificial chromosomes and describes the results

of each step so that one of skill in the art need only follow the teachings to reproducibly and "predictably" obtain satellite artificial chromosomes. The application further describes the results of extensive studies of the replication of satellite artificial chromosomes, which revealed the existence of a higher-order replication unit (megareplicon) of the pericentric region. The megareplicon is delimited by a primary replication initiation site (megareplicator), and appears to facilitate replication of the centromeric heterochromatin, and, most likely, centromeres. Integration of heterologous DNA into the amplifiable megareplicator region or in close proximity thereto, initiates a large-scale amplification of megabase-size chromosomal segments, which leads to *de novo* chromosome formation.

As discussed below, the specification describes in great detail methods for generation of satellite artificial chromosomes. A method includes the steps of introduction of DNA fragments into cells, which are then cultured, identification of structures indicative of the requisite amplification event, and selection of cells that contain a satellite artificial chromosome or precursors thereof. As discussed above, the structures of these chromosomes are characterized and described in exquisite detail in the specification such that of skill in the art could readily recognize and select cells containing them.

The Applicant has undertaken a painstaking analysis of each of the steps of a method for generating the satellite artificial chromosomes, characterizing each of the intermediate structures including dicentric / multicentric chromosomes, formerly dicentric chromosomes or sausage chromosomes in exquisite detail. The characterization of the intermediates involved in the generation of the intermediates described in the specification provides an understanding of the mechanism underlying the method of satellite artificial chromosome formation. Although the selection of cells containing satellite artificial chromosomes does not necessitate prior isolation and selection of cells containing precursor or intermediate species, the detailed description and

characterization of the intermediate species in the specification further demonstrates that Applicant had possession of satellite artificial chromosomes at the time the application was filed, and that these chromosomes could be readily and "predictably" reproduced and identified.

The application describes how cells containing satellite artificial chromosomes are selected. For example, at page 8, line 18 to page 9, line 8 of the specification:

> **Also provided are SATACs of various sizes that are formed by repeated culturing under selective conditions and subcloning of cells that contain chromosomes produced from the formerly dicentric chromosomes.** The exemplified SATACs are based on repeating DNA units that are about 15 Mb [two ~7.5 Mb blocks]. The repeating DNA unit of SATACs formed from other species and other chromosomes may vary, but typically would be on the order of about 7 to about 20 Mb. The repeating DNA units are referred to herein as megareplicons, which in the exemplified SATACs contain tandem blocks of satellite DNA flanked by non-satellite DNA, including heterologous DNA and non-satellite DNA. Amplification produces an array of chromosome segments [each called an amplicon] that contain two inverted megareplicons bordered by heterologous ["foreign"] DNA. **Repeated cell fusion, growth on selective medium and/or BrdU [5-bromodeoxyuridine] treatment or other treatment with other genome destabilizing reagent or agent, such as ionizing radiation, including X-rays, and subcloning results in cell lines that carry stable heterochromatic or partially heterochromatic chromosomes, including a 150-200 Mb "sausage" chromosome, a 500-1000 Mb gigachromosome, a stable 250-400 Mb megachromosome and various smaller stable chromosomes derived therefrom.** These chromosomes are based on these repeating units and can include heterologous DNA that is expressed. (emphasis added)

Thus, satellite artificial chromosomes of various sizes are formed by repeated culturing under selective conditions and subcloning of cells that contain chromosomes produced from the formerly dicentric chromosomes.

Further, at page 34, lines 8-27, the specification, alluding to Figure 2 that elucidates a step-by-step procedure for the generation of SATACs, describes the

production and identification of megachromosomes and gigachromosomes, which, as discussed above, are types of SATACs:

> In particular, if a cell with a dicentric chromosome is selected, it can be grown under selective conditions, or, preferably, additional DNA encoding a second selectable marker is introduced, and the cells grown under conditions selective for the second marker. **The resulting cells should include chromosomes that have structures similar to those depicted in Figures 2D, 2E, 2F. Cells with a structure, such as the sausage chromosome**, Figure 2D, can be selected and fused with a second cell line to eliminate other chromosomes that are not of interest. If desired, cells with other chromosomes can be selected and treated as described herein. **If a cell with a sausage chromosome is selected, it can be treated with an agent, such as BrdU, that destabilizes the chromosome so that the heterochromatic arm forms a chromosome that is substantially heterochromatic [i.e., a megachromosome, see, Figure 2F]. Structures such as the gigachromsome in which the heterochromatic arm has amplified but not broken off from the euchromatic arm, will also be observed.** The megachromosome is a stable chromosome. Further manipulation, such as fusions and growth in selective conditions and/or BrdU treatment or other such treatment, can lead to fragmentation of the megachromosome to form smaller chromosomes that have the amplicon as the basic repeating unit. (emphasis added)

Furthermore, as discussed above, the specification describes the exact procedures used repeatedly to generate multiple specific cell lines containing satellite artificial chromosomes. For example, the specification describes that the G3D5 and H1D3 cell lines were obtained by treating 19C5xHa4 cells with BrdU, followed by growth in either G418 or hygromycin-containing selective medium, respectively, followed by additional BrdU treatments, and the cell lines were characterized by identification of the megachromosomes carried by them (page 39, lines 6-24 of the specification). Generation and characterization of these cell lines comprise growing the cells under conditions that produce the satellite artificial chromosomes, and identifying cells containing satellite artificial chromosomes that fit the descriptions detailed in the specification.

The specification also describes methods of inserting heterologous DNA into satellite artificial chromosomes and minichromosomes and the expression of

the heterologous DNA contained therein in cells (*see, e.g.*, page 39, line 25, through page 40, line 21; page 48, lines 1-10; and Example 12 beginning on page 140). Procedures for the isolation of artificial chromosomes (*see, e.g.*, page 41, line 4 through page 42, line 3; and Example 10, beginning on page 124) and for the transfer of artificial chromosomes into cells (*see, e.g.*, page 10, lines 25-31; page 11, lines 14-18; page 33, lines 25-28; page 47, line 24 through page 53, line 7; and page 53, lines 20-29) are also described in detail in the specification. As discussed above, the specification details isolation, purification of SATACS and transfer of SATACs into cells (*e.g.*, page 41 *et seq.* and EXAMPLE 10).

Thus, the specification describes in great detail the structural and functional characteristics of satellite artificial chromosomes, including plant satellite artificial chromosomes, so that they are readily identified, cell lines containing the satellite artificial chromosomes, methods for generating satellite artificial chromosomes and for introducing the artificial chromosomes into cells, and methods of identifying satellite artificial chromosomes and intermediates thereof. Therefore, it is respectfully submitted that Applicant had possession of the claimed subject matter at the time the instant application was filed and as of its earliest priority date (see, *e.g.*, issued U.S. Patent Nos. 6,077,697 and 6,025,155, which include claims directed to isolated SATACS, cells containing the SATACS, and methods of making the SATACS).

The enablement rejection

Claims 50-52 stand rejected under 35 U.S.C. § 112, first paragraph, because the written description requirement allegedly is not met, one of skill in the art would not know how to make or use the claimed subject matter. Further, the Office Action reiterates the reasons of record for inadequate scope of enablement as set forth in the Office Action of January 17, 2003. It is alleged that the specification, while being enabling for a mammalian satellite artificial chromosome in a mammalian cell, does not reasonably provide

enablement for the introduction of a satellite artificial chromosomes of any species in any cell.

In particular, the Office Action alleges (1) that the differences between plants and animals, particularly their respective satellite DNA, centromeres and heterochromatin, make it unpredictable that a plant would have centromeres that are structurally and biochemically the same as those of animals; and (2) that the alleged complexity of satellite artificial chromosomes, especially their large sizes, when combined with other components of heterologous expression constructs such as different promoters, enhancers, codon optimization, termination regions and other regulatory regions, would lead one of skill in the art to expect that a satellite artificial chromosome constructed for mammalian cells would differ from a satellite artificial chromosome that is constructed for a plant cell. The Office Action further alleges that while the specification provides methods for the preparation and transfer of an animal satellite artificial chromosome into a mammalian cell (human, mouse and hamster cells), there is no evidence that these methods produce a satellite artificial chromosome from any source (*e.g.*, plants) that is operable in any cell type (*e.g.*, a plant cell).

The Examiner states that the DECLARATION of Fabijanski filed responsive to the previous Office Action (hereinafter, DECLARATION I) has been thoroughly considered but is allegedly not persuasive because (1) the DECLARATION states that SATACs were introduced into tobacco using microcells prepared according to a procedure described in U.S. patent No. 6,077,697, which is a post-filing reference that fails to demonstrate that the disclosure was enabling as of its filing date; and (2) the DECLARATION allegedly lacks any teaching of a plant SATAC, a plant SATAC in a plant cell, a plant SATAC in a plant or a plant SATAC in an animal cell. The Examiner concludes that "even after consideration of the DECLARATION, one would not be reasonably apprised of what components make up a SATAC, or a plant SATAC."

It is alleged that while one of skill in the art can readily make changes to Applicant's animal satellite artificial chromosomes to generate a non-animal satellite artificial chromosome, there is no guidance as to what these changes should be, leading one of skill in the art to make random changes fraught with trial and error, requiring undue experimentation. The Office Action concludes that the specification is not enabling for a satellite artificial chromosome that is universally adapted to be operable in all cell types.

This rejection is respectfully traversed.

Relevant law

To satisfy the enablement requirement of 35 U.S.C § 112, first paragraph, the specification must teach one of skill in the art to make and use the invention without undue experimentation. Atlas Powder Co. v. E.I. DuPont de Nemours, 750 F.2d 1569, 224 USPQ 409 (1984). This requirement can be met by providing sufficient disclosure, either through illustrative examples or terminology, to teach one of skill in the art how to make and how to use the claimed subject matter without undue experimentation. This clause does not require "a specific example of everything *within the scope* of a broad claim." In re Anderson, 176 USPQ 331, at 333 (CCPA 1973), emphasis in original. Rather, the requirements of § 112, first paragraph "can be fulfilled by the use of illustrative examples **or** by broad terminology." In re Marzocchi et al., 469 USPQ 367 (CCPA 1971)(emphasis added).

Further, because "it is manifestly impracticable for an applicant who discloses a generic invention to give an example of every species falling within it, or even to name every such species, it is sufficient if the disclosure teaches those skilled in the art what the invention is and how to practice it." In re Grimme, Keil and Schmitz, 124 USPQ 449, 502 (CCPA 1960). Thus, there is no doubt that a patentee's invention may be broader than the particular embodiment shown in the specification. A patentee not only is entitled to narrow claims particularly directed to the preferred embodiment, but also to

broad claims that define the invention without a reference to specific instrumentalities. Smith v. Snow, 294 U.S. 1, 11, 24 USPQ 26, 30 (1935).

The inquiry with respect to scope of enablement under 35 U.S.C. §112, first paragraph, is whether it would require undue experimentation to make and use the claimed invention. A considerable amount of experimentation is permissible, particularly if it is routine experimentation. The amount of experimentation that is permissible depends upon a number of factors, which include: the quantity of experimentation necessary, the amount of direction or guidance presented, the presence or absence of working examples, the nature of the invention, the state of the prior art, the relative skill of those in the art, the predictability of the art, and the breadth of the claims. Ex parte Forman, 230 USPQ 546 (Bd. Pat. App. & Int'f 1986); see also In re Wands, 8 USPQ2d 1400 (Fed. Cir. 1988).

Analysis

Summary of points addressed

1) Responsive to the Examiner's assertion that the instant claims are not supported by an enabling disclosure because the claimed subject matter lacks written description, it is respectfully submitted that the written description and enablement requirements are not coterminus with respect to one another. As held in Vas-Cath, Inc. v. Mahurkar, 935 F.2d at 1563-64, 19 USPQ2d at 1117, the purpose of the written description requirement is to demonstrate that Applicant had possession of the claimed subject matter as of its earliest filing date, and this requirement goes beyond a mere explanation of how to "make and use" the claimed subject matter. Moreover, as discussed above with written description requirement, the specification describes in great detail the claimed methods and its elements, including detailed characteristics of a SATAC. Therefore, regardless of its inaptness, the rejection of the instant claims for lack of enablement due to lack of written description, fails.

2) Responsive to the Examiner's assertion that the application does not provide any identifiable components of a SATAC, including a plant SATAC, as discussed above with respect to the written description requirement, the application provides the identifying features and components of a SATAC, regardless of its species (plant or animal), in great detail. The specification provides detailed descriptions of the structural and functional characteristics of satellite artificial chromosomes, including plant satellite artificial chromosomes, so that they are readily identified. Further, several exemplary SATACs and cell lines containing the SATACs are provided. Furthermore, methods for generating satellite artificial chromosomes of any species and for introducing the artificial chromosomes into any cells, including plant cells such as protoplasts, methods of identifying satellite artificial chromosomes and intermediates thereof, and methods for generating transgenic plants from plant cells or protoplasts into which satellite artificial chromosomes have been introduced, are provided.

The instant application teaches a broad method for introduction of SATACs into cells, which is not limited by the species of SATAC or species of cells, and the generation of transgenic animals or plants therefrom. As the Examiner has acknowledged, the specification is enabling for the introduction of mammalian SATACs into mammalian cells.

Further, although not needed, the DECLARATION I of Fabijanski filed responsive to the previous Office Action demonstrates that by following the teachings of the specification, one of skill in the art can transfer a mammalian SATAC into a plant cell using a variety of transfer techniques and a variety of plant cells. The DECLARATION I provides a SATAC generated according to the teachings of the specification, and demonstrates its successful transfer into a plant cell. DECLARATION I further demonstrates that SATACs of one species (*e.g.*, mammal) acn be delivered into cells of another species (*e.g.*, plant). Also, as discussed below, the DECLARATION submitted herewith demonstrates that by following the teachings of the application, a plant SATAC can be generated.

Therefore, Applicant respectfully submits that the instant application teaches how to make and use subject matter that is commensurate with the scope of the claims.

3) Furthermore, although not needed, Applicant submits herewith a DECLARATION of Fabijanski (DECLARATION II) demonstrating that by following the teachings of the instant application, plant SATACs can be generated and can be stably maintained in plant cells. Therefore, it is respectfully submitted that, contrary to the Examiner's assertions, by following the teachings of the specification, one of skill in the art can identify and generate a SATAC, including a plant SATAC, and the SATACs can further be introduced into animal or plant cells. In addition, by following the teachings of the specification, SATACs of one species (*e.g.*, mammals) can be introduced and stably maintained in cells of a different species (*e.g.*, plants).

4) Applicant also addresses herein specific points raised in the Office Action regarding arguments made in the response of July 16, 2003, in connection with teachings of the specification, knowledge of those of skill in the art, presence of working examples, predictability and DECLARATION I. These points focus on a number of issues: the ability to recognize elements of SATACs and plant SATACs; exemplification of plant SATACs and components of plant SATACs; exemplification of transfer of DNAs into plant cells; alleged differences between plant and animal SATACs; and operability of the claimed methods as assessed by post-effective filing date references. Each of these issues are addressed below.

1. Application of the factors enumerated in <u>In re Wands</u> for enablement

The inquiry with respect to scope of enablement under 35 U.S.C. §112, first paragraph, is whether it would require undue experimentation to make and use the claimed invention. As discussed in detail below, Applicant respectfully submits that the instant application teaches the introduction of a SATAC, including a plant SATAC, into a cell, including plant cells or protoplasts, and the

generation of a transgenic plant therefrom. It would not require undue experimentation to introduce a SATAC of any species into a cell of any species, including a protoplast, nor would it require undue experimentation to produce a plant SATAC, a plant cell with a SATAC or a cell with a plant SATAC. Further, as discussed above with respect to the written description requirement, the SATACs, including plant SATACs, are described and characterized in the specification in great detail. Therefore, one of skill in the art can readily identify SATACs, plant SATACs and cells containing SATACs without undue experimentation.

The instant application teaches SATACs, including plant SATACs, and methods of producing the SATACs. The application also teaches introduction of SATACs into different cell types, including plant cells, as claimed. Further, production of SATACs has been established and Applicant has been granted claims to SATACs and methods of producing SATACs in issued patents U.S. Patent Nos. 6,077,697 and 6,025,155, which are based on the same parent applications as the instant application.

As demonstrated below, the teachings of the specification are such that it would not require undue experimentation to perform the steps of the methods claimed herein, including the preparation and isolation of animal or plant satellite artificial chromosomes, their introduction into a plant cell such as a plant protoplast of any species and the growth of the plant cell under conditions to produce transgenic plants containing a satellite artificial chromosome.

Scope of the Claims

The claims are directed to methods for producing a transgenic plant by introducing a satellite artificial chromosome into a plant protoplast and growing the plant protoplast under conditions to produce a transgenic plant. The dependent claims specify that the satellite artificial chromosomes comprise heterologous DNA that encodes a gene product; and particular methods for the introduction of the satellite artificial chromosomes into plant protoplasts.

Level of Skill

The level of skill in this art is recognized to be high (see, *e.g.*, Ex parte Forman, 230 USPQ 546 (Bd. Pat. App. & Int'f 1986). The numerous articles and patents made of record in this application address a highly skilled audience and further evidence the high level of skill in this art. Therefore, Applicant respectfully submits that using the teachings of the specification, one of skill in the art, could apply such teachings to making non-animal SATACs, such as plant SATACs, introducing SATACs into numerous cell types, including plant cells and protoplasts, and generating transgenic plants therefrom.

Teachings of the specification

Applicant respectfully submits that SATACs and plant SATACs are taught by the instant application. As discussed above with respect to the written description requirement, the specification provides descriptions of SATACs and plant SATACs and structural elements thereof. The specification further teaches the exact steps involved in generating SATACs and describes the results of each step so that one of skill in the art need only follow the teachings to obtain SATACs of any species. It is only necessary to introduce a piece of DNA and a selectable marker into a cell, grow the cell under selective conditions, look for cells that contain satellite artificial chromosomes, select such cells, and isolate a satellite artificial chromosome therefrom. The specification also teaches the introduction of SATACs of any species into any cells, including plant cells such as protoplasts, and the generation of transgenic plants therefrom.

1) Production of SATACs including Plant SATACs

The instant application teaches SATACs and methods of making SATACs. As stated in the instant application, the methods provided can be applied to different species (page 12, lines 2-3). Additionally, Applicant is not required to teach what is well known in the art. In re Buchner, 929 F.2d 660, 661, 18 USPQ2d 1331, 1332 (Fed. Cir. 1991). Thus, it is sufficient if one of skill in the

art can make and use the claimed subject matter using the teachings of the specification coupled with information known in the art. MPEP § 2164.01.

A SATAC, like most chromosomes, is a large structure. In contrast to an individual gene where the DNA sequence is the essence of the gene, it is structure that is the essence of a SATAC. The instant application teaches the structure of a SATAC (see, for example, pages 44-47 and Figures 2-3). The specification further teaches identifying elements of a SATAC, *e.g.*, centromere, telomeres, origin of replication, and heterochromatin.

The teachings of the specification are not limited to animal SATACs, but provide SATACS, including SATACs from any species, plant or animal. The specification teaches that a plant SATAC is a SATAC with a plant centromere (page 16, lines 18-25). The teachings of the specification regarding SATAC structure and elements of SATACs are applicable across species. Further, the structure of chromosomes and elements of chromosomes, such as those found in SATACs, are conserved between species and across kingdoms; centromeres, telomeres, origins of replication and heterochromatin are recognizable structures across species. The specification also teaches methods known in the art for identifying chromosome structure and elements thereof, such as chromosome banding (pages 72-73), immunolabeling, immunofluorescence (page 73) and scanning electron microscopy (page 73), that are applicable to SATACs of any species.

Applicant respectfully submits that one of skill in the art could use the teachings of the instant application with what was known as of the effective filing date to practice the claimed methods, including producing plant SATACs. The specification describes how to make SATACs and exemplifies the formation of SATACs such that one of skill in the art would be able to follow the teachings to generate plant SATACs. It is only necessary to introduce heterologous DNA, generally with a selectable marker (to aid in indentifying cells in which an amplification event has occurred) into a cell, grow the cell, under

selective conditions if a selectable marker is included, look for cells that contain satellite artificial chromosomes, select such cells. The satellite artificial chromosome can be isolated from the cells. There is no evidence of record that suggests that such events are unique to animal cells, nor is there any reason to believe such. On the contrary, the specification teaches that the events and the methods based thereon are applicable to cells from any eukaryotic species. As noted above and as discussed responsive to the previous Office Action, the generation of a satellite artificial chromosome requires an amplification event and there is no reason provided by the Examiner nor of record that suggests that plant chromosomes do not undergo amplification. In fact, as discussed earlier, plants have amplifiable regions (see, *e.g.*, U.S. Patent Nos. 6,355,860 and 6,100,092, which demonstrate this assertion; see, also Borysuk *et al.* (1988) *Theor. Appl. Genet 76*:108-112 and Borysuk *et al.* (1993) *Plant Mol. Biol. 21*:381-384). Further, as discussed below, the DECLARATION II of Fabijanski submitted herewith demonstrates that by following the method taught by the instant application for the generation of a SATAC, one of skill in the art can in fact generate a plant SATAC.

The specification teaches identification and characterization of the satellite artificial chromosomes and each of the intermediates generated in the process of *de novo* satellite artificial chromosome formation. Extensive analyses using methods including Southern hybridization, long-range mapping of restriction endonuclease sites, indirect immunofluorescence with anti-centromere antibodies provide definition of the artificial chromosomes at the level of the basic structural and functional elements that comprise these chromosomes, including the characteristic repeated units of satellite and foreign DNA. Many of these features are depicted schematically in Figures 1-3 of the application.

For example, the specification exemplifies generation of SATACs. As exemplified, the amplification event leads to formation of a sausage chromosome from a formerly dicentric chromosome (see for example, Example

4, pages 83-91, and Figures 2). The specification exemplifies formation of a megachromosome, which is a type of satellite artificial chromosome, from a sausage chromosome and teaches the structural features of the megachromosome observed with immunofluorescence and with electron microscopy (see for example, Example 6, pages 92-97). Events leading to formation of a SATAC occur by following the teachings of the specification (*i.e.*, introducing heterologous nucleic acid into a cell, growing the cell and identifying cells in which chromosomes have undergone amplification).

By following these teachings, one of skill in the art can produce and recognize a SATAC from any species, including a plant and produce and recognize a SATAC. Furthermore, at the time of effective filing date of this application, there was a lot of knowledge in the art regarding plant chromosomes and elements of chromosomes such that the teachings of the specification those of skill in the art could readily produce SATACs from any species including plant SATACs based upon the teachings in the specification.

As discussed, the specification provides methods of generating satellite artificial chromosomes (SATACs), and characterizes in exquisite detail the artificial chromosomes generated by such methods. To illustrate the methods and products thereof, the specification describes exact procedures used to generate multiple specific cell lines containing SATACs (see, *e.g.*, Examples 2-7, beginning at page 75, line 8), and Applicant provides to the public no less than six of the described cell lines that have been deposited at an authorized depository (*i.e.*, the European Collection of Animal Cell Culture) (see, *e.g.*, page 74, line 23, through page 75, line 7).

The specification teaches the development of a satellite artificial chromosome containing sequences that can be of animal or plant origin and contain centromere-related sequences. For example, the specification provides methods for generating species-specific satellite artificial chromosomes by

adding a centromere from other species, including plants (see, *e.g.*, at page 12, lines 3-16).

In the specification, *e.g.*, at page 11, line 30, through page 12, line 16, a method for cloning a centromere in a selected species (*e.g.*, plants) is described. These methods for cloning centromeres from a selected animal or plant include: (i) preparing a library of DNA fragments that contains the genome of the plant or animal; (ii) introducing each of the fragments into a mammalian satellite artificial chromosome that contains a selectable marker and a centromere from a species different from the selected plant or animal; (iii) introducing each of the satellite artificial chromosomes into a cell, which is grown under selective conditions; and (iv) selecting cells containing satellite artificial chromosomes. Satellite artificial chromosomes that are identified by the methods provides herein should contain a centromere encoded by the DNA from the library, be it plant DNA or mammalian DNA, and should contain the necessary elements for stable replication in the selected species. Thus, the specification provides methods where a satellite artificial chromosome is developed in one source cell, modified to contain sequences specific to the centromere region of a target cell and then transferred to the target cell type.

Methods for the modification of satellite artificial chromosomes are described in extensive detail in the specification (*e.g.*, at page 39, line 26, through page 40, line 21; and page 150, through page 157). The cited passage(s) describe the use of homologous recombination to insert new DNA fragments into a satellite artificial chromosome. Accordingly, methods to generate and modify a satellite artificial chromosome to contain a plant centromere DNA sequences have been provided.

The specification also teaches additional methods for identifying and constructing elements of SATACs. For example, at page 12, lines 3-12, the specification teaches methods of isolating centromeres by using probes that recognize centromere sequences and further at page 12, lines 21-29, methods

to assess centromere function are taught. The specification also describes the construction of telomeres and satellite repeats (see for example, pages 64-68). One of skill in the art could assemble a plant SATAC using these components and furthermore, as discussed above, recognize the SATAC as obtained by the descriptions of SATACs as described in the specification.

Therefore, the specification teaches one of skill in the art how to generate SATACS from various species (*e.g.*, plants and animals); readily identify the resulting satellite artificial chromosomes based on the detailed characterization provided in the specification; incorporate foreign nucleic acid (*e.g.*, heterologous DNA encoding a product into an artificial chromosome) and isolate and transfer artificial chromosomes into cells from various species (*e.g.*, plants and animals). Thus, the teachings of the specification provide how to make and use the satellite artificial chromosomes and to combine these artificial chromosomes with known recombinant DNA procedures, many of which are referenced in the specification, to achieve any number of particular outcomes, including the introduction and long-term expression of nucleic acids encoding products in cells of host animals, plants and insects.

The applicability of the teachings of the specification for making and identifying plant SATACs is further evidenced by the DECLARATION II, provided herein. The DECLARATION II demonstrates that by following the teachings of the specification, one of skill in the art can generate a plant SATAC, which is identified by its features as taught in the specification. For example, as shown by the DECLARATION II, one can introduce heterologous DNA with homology to pericentric DNA to generate a chromosome with a "sausage amplification" and a plant SATAC. The plant SATAC generated is recognized by structural features of SATACs as described in the specification, such as the identification of a plant centromere and the presence of heterochromatin.

2) Introduction of SATACs into cells

The specification teaches numerous methods for introducing SATACs into a cell. Such methods include direct DNA transfer, electroporation, lipid-mediated transfer, *e.g.*, lipofection and liposomes, microprojectile bombardment, and microinjection (see for example, pages 48-56). The specification teaches that chromosomes can be transferred by preparing microcells containing an artificial chromosome and then fusing with selected target cells. Cell fusion can be used to transfer SATACs (see for example, page 53, lines 23 and page 54, line 6). Cell fusion is also exemplified in Example 1.

The specification further teaches methods for producing satellite artificial chromosomes that contain heterologous DNA and the expression of the heterologous DNA contained therein in cells (see, *e.g.*, page 39, line 25, through page 41, line 3; page 61, line 28, through page 62, line 7; page 150, line 1, through page 165, line 12 and Example 12 beginning on page 140). Procedures for the isolation of artificial chromosomes (see, *e.g.*, page 41, line 4, through page 42, line 3; page 32, lines 13-24; page 80, lines 20-27; and Example 10, beginning on page 124) and for the transfer of the artificial chromosomes into cells (see, *e.g.*, page 10, lines 25-31; page 48, line 11, through page 51, line 26; page 52, line 11, through page 55, line 3; page 70, line 14, through page 72, line 27; and Example 13 beginning on page 165) are also described in detail in the specification.

At page 54, line 1, through page 55, line 3, the specification describes how to introduce SATACs into plant cells by methods, such as direct transfer of DNA by processes, such as PEG-induced DNA uptake, protoplast fusion, microinjection, electroporation, and microprojectile bombardment, such as particle gun bombardment. Further, as the Office Action acknowledges at page 10, "The Office Action does not contest that once one has a plant SATAC, transformation of plant cells was in the skill of the art." Therefore, Applicant respectfully submits that, as the Examiner has acknowledged, one of skill in the

art could use methods available in the art to introduce a SATAC of any species into any cell, including a plant cell.

The DECLARATION I submitted responsive to the previous Office Action further demonstrates that the teachings of the specification provide adequate guidance for introducing SATACs into plant cells. As shown in the DECLARATION I, SATACs can be introduced into a variety of plant cells using techniques taught in the instant application, such as microcell-mediated cell fusion and lipid-mediated transfection.

3) Generation of transgenic plants

Methods of producing a transgenic plant from a plant cell such as a plant protoplast were well known to those of skill in the art at the time of filing of the above-captioned application and as of its earliest priority date. As the specification recites, the method used for producing a transgenic plant is primarily a function of the species of plant (exemplary species of plant are provided in the specification, see, *e.g.*, page 54, lines 12-16) and protocols used to introduce DNA into plant cells and produce a transgenic plant are readily adapted by one of skill in the art. Further, the specification incorporates by reference, methods of producing transgenic plants (see, *e.g.*, U.S. Patent Nos. 5,489,520 and 5,482,928).

Thus, the teachings of the specification provide how to make and use the satellite artificial chromosomes and to combine these artificial chromosomes with known recombinant DNA procedures, many of which are referenced in the specification, to achieve any number of particular outcomes, including the introduction and long-term expression of satellite artificial chromosomes in eukaryotic cells, including plant cells, and the generation of transgenic plants from these cells.

Knowledge of those of skill in the art

As of the effective filing date of the application, a broad body of knowledge was available about properties and structural components of chromosomes. Such knowledge included availability of structural and sequence information for plant centromeres, telomeres, and satellite DNA. Methods of DNA transfer into cells and between different cell types were available. Also known to those of skill in the art were methods for the manipulation of DNA, recombinant DNA techniques, techniques for the transfer of DNA into cells, including plant cells, and the production of transgenic plants from plant cells such as protoplasts containing heterologous DNA. Many examples of such knowledge are cited in the instant application, in the instant response and in the response of July 16, 2003.

Elements of SATACs, identified in the instant application, were available in the art for plant SATACs. For example, sequence information for plant centromeres, telomeres and autonomously replicating sequences (ARS) was available (see, *e.g.*, ; Richards, E.J. "Plant Telomeres" in *Telomeres* Eds. C. Greider, and E.H. Blackburn, Cold Spring Harbor Laboratory Press, Cold Spring Harbor (1995); Rhodes *et al. Curr. Opin. Struct. Biol. 5(3):* 311-322 (1995); Zakian *Science 270(5242):* 1601-1607 (1995); Berlani *et al. Plant Mol. Biol. 11:* 161-162 (1988); Berlani *et al. Plant Mol. Biol. 11:* 173-182 (1988); and Eckdahl *et al. Plant Mol. Biol. 12:* 507-516 (1989)). Pericentric DNA sequences in plant DNA were known; see for example, Genbank Accession no. X52320; submitted 25 April 1990; Schmidt *et al. Science* (1995) 270:480-83; Murata *et al.* (1994) *Jpn. J. Genet.* 69:361-70.

Techniques and materials for plant transformation for use with SATACs were also available in the art. Numerous techniques for introducing DNA into plant cells were known, for example, Uchimiya *et al.* (1989) *J. of Biotech.* 12: 1-20, Weissbach *et al.* (1988) Methods for Plant Molecular Biology, Academic Press, N.Y., Section VIII, pp. 421-463; Grierson *et al.* (1988) Plant Molecular

Biology, 2d Ed., Blackie, London, Ch. 7-9, Miranda *et al.* (1992) *J. Bacteriol.* 174:2288-97 and for example, U.S. Patent Nos. 5,436,392; 5,489,520; 5,470,7085; 491,075; 5,482,928; and 5,424,409. Numerous techniques were also available for use in transferring SATACs from one cell into another cell, for example, Parkonny *et al.*, (1992) *Plant J.* 2:863-74; Constabel F. (1976) *In Vitro* 12: 743-8; Jones *et al.* (1976) *Science* 194:401-03; Cocking (1984) *Ciba Found Symp.* 103:199-28. Plant selectable markers were available, such as phosphinothricin acetyl transferase and hygromycin (see for example, White *et al.* (1990) *Nuc. Acids Res.* 18:1062; Spencer *et al.* (1990) *Theor. Appl. Genet.* 79:625-631; Vickers *et al.* (1996) *Plant Mol. Biol. Reporter* 14:363-368; Thompson *et al.* (1987) *EMBO J.* 6:2519-2523; and Blochinger and Diggelmann, *Mol. Cell. Biol.* 4:2929-2931).

Techniques for identifying SATACs as described in the instant application were available for use with plant SATACs and plant cells. For example, chromosome banding and labeling, Wang & Fedoroff (1972) *Nature 235*:52-54, Sumner (1972) *Exp. Cell Res. 75*:304-306, Perry & Wolff (1974) *Nature 251*:156-158; Immunolabeling, Hadlaczky *et al.* (1986) *Exp. Cell Res. 167*:1-15, Hadlaczky *et al.* (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*:8106-8110; electron microscopy, Sumner (1991) *Chromosoma 100*:410-418. Such techniques were known to one of skill in the art and had also been demonstrated as applicable with plant chromosomes as well as with plant cells (see for example, Sparvoli *et al.* (1994) *J. Cell Science* 107:3097-3103 and Wanner *et al.*(1995) *Chromosome Res.* 3:368-74).

Methods for introducing heterologous DNA into plant cells and generating transgenic plants from the transformed cells were well known in the art at the time of filing and before. For example, as of the earliest priority of the date of the above-captioned application, it was recognized that a variety of plant cells, including plant protoplasts, were versatile tools for introducing heterologous DNA, monitoring gene expression in the transformed cells, conducting

developmental studies and generating transgenic plants. As of the earliest priority date of the above-captioned application, those of skill knew that plant cells could readily be transformed and induced to generate a variety of plants (*see, e.g.*, Negrutiu *et al.*, *Int. J. Dev. Biol.*, 36:73-84 (1992); Rogers *et al.*, "Methods for Plant Molecular Biology," Academic Press (1988) VIII(26):423-436; Zupan *et al.*, *Proc. Natl. Acad. Sci. USA*, 93:2392-2397 (1992)).
As the specification states, the method used for producing a transgenic plant is primarily a function of the species of plant. At the time of filing, numerous protocols were available for producing transgenic plants. The protocols used to introduce DNA into plant cells and produce a transgenic plant are readily adapted by one of skill in the art.

These references to numerous published information and protocols regarding plant and animal chromosomal composition and structure, DNA manipulation, recombinant DNA expression, transfer of DNA into cells, including plants cells, and production of transgenic plants demonstrate the large volume of information regarding tested and reliable procedures available at the time of filing of the instant application and thus evidence the advanced state of the art at the relevant time and the availability of such procedures for manipulation of plant cells, introduction of satellite artificial chromosomes into plant cells and production of transgenic plants from the satellite artificial chromosome-containing plant cells.

Presence of Working Examples

The specification provides numerous working examples and descriptions of the generation, isolation and transfer of SATACs from various sources. Such examples are valuable not only for animal SATACs and SATAC transfer into animal cells, but also for the construction of plant SATACs and SATAC transfer into plant cells.

The working examples of animal SATACs provide sufficient teachings, in combination with what was known to those of skill in the art at the time of the

instant application's earliest priority date, to generate, identify and transfer a SATAC, regardless of the organism from which it is derived or the cell type to which it is introduced. For example, techniques for use in the generation and identification of SATACs are provided in Example 1, such as chromosome banding and labeling methods, cell fusion and microcell fusion methods and DNA methods (see for example, pages 69-74). The specification also provides detailed examples of SATAC formation including descriptions of intermediary structures such as dicentric chromosomes and sausage chromosomes (*see*, for example, Example 4, which describes formation of a sausage chromosome from a formerly dicentric chromosome). The specification provides examples of SATAC production from such intermediates (*see*, for example, Example 6, which describes production of a megachromsome from the sausage chromosomes at pages 92-93), and additionally provides schematic representations of SATAC formation and exemplary SATACs in Figures 2 and 3. Structural features of exemplary SATACs, such as megachromosomes, are also described (*see*, for example, pages 93-97).

Example 2, at page 75 of the specification, describes in great detail the preparation and maintenance of cell lines, including EC3/75, EC3/7C5 and EC3/7C6, which contain artificial chromosomes, as well as the assays used to monitor the expression of the *neo* gene encoded by the artificial chromosomes within the cells. Example 6, at page 92 of the specification, describes in great detail, methods for the generation of cell lines containing a megachromosome and detailed structural characteristics of this satellite artificial chromosome. Example 8, at page 113 of the specification, describes in great detail the *in vivo* replication of a megachromosome. Example 10, at page 124 of the specification, describes in great detail methods for the isolation of satellite artificial chromosomes from endogenous chromosomes based upon the atypical base content and/or size of the satellite artificial chromosome. Example 12, at page 140 of the specification, describes in great detail the preparation of

vectors and plasmids, such as the λCF-7 and the λCF-7-DTA vectors and the pMCT-RUC and the pLNCX-ILRUC plasmids, for the targeted integration of heterologous DNA into artificial chromosomes. Example 13, at page 165 of the specification, describes methods for the microinjection of artificial chromosomes into eukaryotic cells, and detection of expression of the encoded heterologous DNA (βGal) in cells injected with the DNA.

Although the working examples exemplify the teachings in mammalian cells, the teachings are directly applicable to plant cells when placed in the context of the instant application in its entirety, which provides further guidance for plant SATACs and introduction of SATACs into plant cells. Further, Applicant is not required to provide data or illustrative examples in support of every assertion in the specification or everything within the scope of a broad claim. In re Anderson, 176 USPQ 331, at 333 (CCPA 1973)). Nonetheless, as discussed below, the accompanying DECLARATION II demonstrates that by following the teachings of the instant application, which are exemplified in the working examples, one of skill in the art can generate plant SATACs and maintain plant SATACs in plant cells. Further, DECLARATION I accompanying the response to the previous Office Action demonstrates that by following the teachings of the specification, one of skill in the art can introduce into and stably maintain mammalian SATACs in plant cells.

Predictability

Using the teachings of the specification and given the level of skill and the knowledge of those of skill in the art, it would not be unpredictable to make a plant SATAC, nor to transfer a SATAC into a plant cell and generate a transgenic plant therefrom. As discussed above in great detail, the specification teaches methods for making SATACs, methods for identifying SATACs, including the elements of SATACs and exemplary structures, and methods for introducing SATACs into cells and generating transgenic animals and plants

therefrom. As the specification provides and as explained above, these teachings are applicable to non-animal SATACs, including plant SATACs.

The Office Action appears to imply that the methods for making and identifying SATACs as applied to plants are fundamentally different from the methods for animals and that Applicant has not taught these "other" methods. In response, Applicant respectfully submits that the methods for making plant SATACs are not different from making animal SATACs. As discussed above, the specification teaches the exact steps involved in generating satellite artificial chromosomes and describes the results of each step so that one of skill in the art need only follow the teachings to obtain satellite artificial chromosomes of any species.

For example, the structure and elements of plant SATACs are as described for mammalian SATACs, with the exception that plant SATACs have a plant centromere (page 16, lines 23-25). Therefore, using the teachings of the specification and what was known in the art at the effective filing date of the application, one of skill in the art could make and identify a plant SATAC. Further, the specification teaches that by using heterologous DNA, one can produce dicentric chromosomes that are intermediates in the generation of any SATACs, including plant SATACs (*see*, for example, page 29, lines 11-17, and also Example 2). The ability of plants to form dicentric chromosomes was known in the art (*see*, for example, McClintock (1942) *Proc. N.A.S.* 28:458-63). Additionally, pericentric DNA that may be used as a targeting sequence was known for a variety of plants (see for example, Genbank Accession no. X52320; submitted 25 April 1990; Schmidt *et al. Science* (1995) 270:480-83; Murata *et al.* (1994) *Jpn. J. Genet.* 69:361-70). Selectable markers such as phosphinothricin acetyl transferase and hygromycin for use in plants were also known (*see*, for example, White *et al.* (1990) *Nuc. Acids Res.* 18:1062; Spencer *et al.* (1990) *Theor. Appl. Genet.* 79:625-631; Vickers *et al.* (1996) *Plant Mol.*

Biol. Reporter 14:363-368; Thompson *et al.* (1987) *EMBO J.* 6:2519-2523; and Blochinger and Diggelmann, *Mol. Cell. Biol.* 4:2929-2931).

The specification further teaches that one can grow cells under selective conditions to obtain a sausage chromosome from a dicentric chromosome (for example, see page 34, lines 8-15) and also obtain a megachromsome and smaller chromosomes (see for example, page 34, lines 16-27). Such methods are exemplified in Examples 4 and 6 and further, representative structures are depicted in Figures 2 and 3. It was known as of the effective filing date that plant chromosomes could undergo breakage-fusion cycles to generate chromosome rearrangements and that such rearrangements could be recognized using standard cytological techniques (McClintock at page 462). Thus, by following the teachings of the specification, one could produce plant SATACs, for example from dicentric chromosomes, and further, using the teachings of the specification and standard cytological methods, identify the plant SATAC that is produced.

The application also teaches and exemplifies how to recognize SATACs and identify cells containing SATACs and as discussed further below, these teachings could be predictably and interchangeably applied to SATACs of any species, including plants. For example, Example 2, at page 72, line 28 to page 73, line 22, teaches chromosome banding, immunofluorescence and electron microscopy can be used to visualize SATACs. Further at page 91, lines 17-29, Example 4 teaches Southern blotting to identify DNA sequences incorporated into SATACs and the use of reporter genes such as β-galactosidase to monitor expression from DNA integrated into chromosomes. These techniques are further exemplified with megachromosomes in Example 6 (see for example C-banding, page 94 lines 5-10; *in situ* hybridization page 94, line 13-page 95, line 16; scanning electron microscopy, page 97 lines 1-15). Techniques such as these were known in the art to be amenable for use with plant chromosomes

and plant cells (see for example, Sparvoli *et al.* (1994) *J. Cell Science 107*:3097-3103 and Wanner *et al.*(1995) *Chromosome Res. 3*:368-74).

The instant application provides further exemplification and teachings that would allow one of skill in the art to predictably identify a SATAC regardless of the species. For example, Example 4 (pages 83- 91) provides an exemplary sausage chromosome including methods for assessing the presence of satellite DNA, distinguishing the number of centromeres present and assays for DNAs present in the pericentric region. Example 5 (page 92) teaches the use of chromosome painting to detect the transfer of chromosomes from one cell to another. Example 6 (pages 92-110) teaches the construction of an exemplary megachromosome, including the assessment of the level of heterochromatin within the SATAC (see for example, page 94, line 3 to page 95, line 27). As described above, the techniques used in these examples to recognize SATACs are suitable for the identification of plant chromosomes and for use with plant cells. Further, the structures identified by such techniques, such as centromeres, satellite DNAs and structures of dicentric and sausage chromosomes, are structures that could be recognized, as of the instant application's earliest priority date, in plant chromosomes and plant cells (see for example, Vega *et al.* (1994) *Proc. Natl. Acad. Sci. USA 91*:12041-12045; Sparvoli *et al.* (1994) *J. Cell Science 107*:3097-3103 and Wanner *et al.*(1995) *Chromosome Res. 3*:368-74).

In summary, a consideration of the factors enumerated in Ex parte Forman leads to the conclusion that undue experimentation would not be required to produce plant SATACs or to introduce SATACs into different cell types, including plant cells, and generate transgenic plants as claimed. The teachings of the instant application are applicable across species and include both animal and plant SATACs. In view of the level of skill in the art and the teachings and disclosure in the specification, it would not require undue experimentation to perform the steps of the methods as claimed for producing

cells with SATACs, including the preparation and isolation of plant satellite artificial chromosomes and their introduction into a cell of any species, including plant cells, to produce cells containing a satellite artificial chromosome.

REBUTTAL TO SPECIFIC ARGUMENTS SET FORTH IN THE OFFICE ACTION

As discussed above, Applicant respectfully submits that a consideration of the factors enumerated in Ex parte Forman (see also In re Wands, 8 USPQ2d 1400 (Fed. Cir. 1988) leads to the conclusion that the specification teaches one of skill in the art to make and use subject matter that is commensurate with the scope of the claims, without undue experimentation. Applicant additionally addresses in turn each of the specific points raised in the Office Action.

1) The Office Action alleges that the instant application does not sufficiently describe SATACs and elements of SATACs such that one of skill in the art could produce a plant SATAC and know if one had obtained a plant SATAC.

Applicant respectfully submits that the instant application provides sufficient description of SATACs for one of skill in the art to produce a plant SATAC and to identify a plant SATAC. As discussed in detail above with regard to both written description and enablement, SATACs and elements of SATACs are described in detail and exemplified throughout the specification and working examples. SATACs are described by structural features such as centromeres, telomeres, and heterochromatin that were well known in the art for many species, including plants. The instant application provides descriptions and drawings exemplifying these structures (*see*, for example, Figures 2 and 3). Thus, one of skill in the art using such teachings could recognize a SATAC. Further, the instant application provides in great detail each of the steps involved in generating a satellite artificial chromosome, including detailed characterization of the intermediates involved, so that one of skill in the art could produce and identify a plant SATAC *de novo*.

Additionally, the instant application teaches that plant SATACs are SATACs with a plant centromere (page 16, lines 23-25). Thus, one of skill in

the art would recognize that the teachings of the structural features of a SATAC were applicable to plant SATACs. Plant centromeres were known in the art and methods to identify plant centromeres within chromosomes were available (see for example, Murata *et al.* (1994) *Jpn. J. Genet.* 69: 361-70; Maluszynska *et al.* (1991) *Plant J. 1*:159-66, Sparvoli *et al.* (1994) *J. Cell Science* 107:3097-3103 and Schmidt *et al.* (1995) *Science 270*:480-483 and Wanner *et al.*(1995) *Chromosome Res.* 3:368-74). Therefore, using the teachings of the specification, one could recognize a plant SATAC.

The instant Application also teaches methods of making SATACs and plant SATACs that can be used in the claimed methods. As discussed in detail with regards to the factors enumerated in Ex parte Forman, the methods taught in the application are applicable to SATACs regardless of species. The working examples and descriptions throughout the specification provide sufficient detail for one of skill in the art to make a plant SATAC using the knowledge in the art available at the time of filing.

Furthermore, the instant application is based on the same parent application as are U.S. Patent Nos. 6,025,155 and 6,077,697, which have issued claims to SATACs and methods of making SATACs. Both patents are incorporated by reference in their entirety in the instant application. The patents teach and claim methods of making SATACs; the claims are not limited by species. For example, claim 1 of 6,077,097 recites:

> A method comprising:
>
> introducing one or more DNA fragments into a cell, wherein the DNA fragment or fragments comprise a selectable marker;
> growing the cell under selective conditions to produce cells that have incorporated the DNA fragment or fragments into their genomic DNA; and
> selecting a cell that comprises a satellite artificial chromosome.

Thus, methods for making SATACs were enabled as of the earliest priority date of the instant application. Further, U.S. Patent No. 6,077,097, includes composition claims to SATACs. For example, claim 8 recites "An isolated substantially pure satellite artificial chromosome." Thus, since an issued patent is presumptively valid, 35 U.S.C § 282, claims of an issued patent are presumptively enabled. Therefore, as of the effective filing date of the instant application, SATACs of any species could be made, identified and isolated using the methods and compositions taught by U.S. Patent Nos. 6,077,097, and 6,025,155.

2) The Office Action also alleges that since one method of producing SATACs includes the use of a selectable marker and a selectable marker is not part of the claims, it is not possible to screen for plant SATACs and identify a SATAC within a cell.

Applicant respectfully submits that, as discussed above and as described and taught in the specification, a selectable marker is not necessary to practice all of the claimed methods. Although selectable markers can be used in the methods of generating SATACs, they are not required (*see*, for example, page 7, lines 6-14 and page 10, lines 5-10). The events leading to formation of a SATAC are not dependent upon use of a selectable marker. As defined on page 16 (lines 18-25), SATACs including plant SATACs do not necessarily include a selectable marker, only the capacity to accommodate heterologous sequences. Further, as described on page 48-56, introduction of SATACs into cells does not require a selectable marker. Thus, Applicant respectfully submits that use of a selectable marker is not essential to practice the claimed methods. In addition, as demonstrated by DECLARATION I filed responsive to the previous Office Action and DECLARATION II provided herewith, SATACs can be introduced into plant cells and/or generated and maintained in plant cells without the use of a selectable marker.

Furthermore, as discussed above, as of the instant application's filing date and earlier, plant selectable markers, such as phosphinothricin acetyl transferase and hygromycin (see for example, White *et al.* (1990) *Nuc. Acids Res.* 18:1062; Spencer *et al.* (1990) *Theor. Appl. Genet.* 79:625-631; Vickers *et al.* (1996) *Plant Mol. Biol. Reporter* 14:363-368; Thompson *et al.* (1987) *EMBO J.* 6:2519-2523; and Blochinger and Diggelmann, *Mol. Cell. Biol.* 4:2929-2931) were known and available. Therefore, it is respectfully submitted that even in the methods that employ selectable markers, it was possible to screen for plant SATACs within a cell as of the application earliest filing date. As noted, since the SATAC itself is an identifiable phenoptype, a selectable marker is not need for identification or production of a SATAC in any species.

3) The Office Action contends that because Applicant has not exemplified plant SATACs, elements of plant SATACs, nor provided any working example of plant SATACs, the scope of the claims is not fully enabled.

Applicant respectfully submits that it is not required to exemplify every embodiment of the claimed subject matter. In re Anderson, 176 USPQ 331, at 333 (CCPA 1973), see also MPEP § 2164.02). Further, as discussed above, the structural elements and functional characteristics of the SATACs and the methods for making SATACs and introducing SATACs into cells are taught in great detail as being applicable to any species. As discussed in detail with regards to predictability, using the teachings of the specification and the knowledge in the art as of the effective filing date, one of skill in the art could make a plant SATAC and produce cells with plant SATACs. The elements of SATACs, in combination with knowledge of plant chromosomes and plant transformation techniques known in the art, could be used with the methods as taught by the instant application. This assertion is further evidenced by the DECLARATION II provided herewith, demonstrating that the teachings of the instant application are operable for making a plant SATAC.

4) The Office Action further alleges that the transfer of SATACs into plant cells is not enabled because Applicant has not exemplified any transfers into plant cells.

The specification teaches the introduction of heterologous DNA into plants using a variety of methods known in the art such as direct transfer of DNA by PEG-induced DNA uptake, protoplast fusion, microinjection, electroporation, and microprojectile bombardment (page 54, lines 4-11). Additional methods of introducing DNA into plants were known in the art as of the effective filing date of the application, including agrobacterium-mediated transformation of large DNAs into plants (*see*, for example, Miranda *et al.* (1992) *J. Bacteriol.* 174:2288-97). It was also known in the art that DNA, including chromosomes, could be transferred by cell fusion. Such cell fusions include fusions between cells of different kingdoms such as between animal and plant cells, as well as transfer between different species within a kingdom (see for example, Parkonny *et al.*, (1992) Plant J. 2:863-74; Constabel F. (1976) *In Vitro 12*: 743-8; Jones *et al.* (1976) Science 194:401-03; Cocking (1984) *Ciba Found Symp. 103*:199-28).

Further, as demonstrated by the DECLARATION I provided responsive to the previous Office Action, using the teachings of the specification, SATACs can be introduced into plant cells using microcell-mediated cell fusion or lipid-mediated transfection. The DECLARATION I further demonstrates that SATACs of one species can be introduced into cells of another species. The DECLARATION I demonstrates, for example, that SATACs derived from murine chromosomes can be introduced and maintained in plant cells such as rice, Arabidopsis and tobacco cells.

5) The Office Action alleges that plants and animals differ such that one would not be able to predictably identify elements of a plant SATAC nor make a plant SATAC.

The Office Action has premised a number of the arguments on the assertion that because plants and animals are in different kingdoms, it necessarily follows that any teachings with respect to animal satellite artificial chromosomes have no bearing on plant satellite artificial chromosomes. Applicant respectfully submits that this assertion is not supported by the references made of record in response to the Office Action of January 17, 2003, or the instant Office Action. There is no suggestion in any of these references that chromosome structure and function is different between plants and animals.

The Office Action alleges that because animals and yeasts have AT-rich satellite DNA and plant satellite DNA is GC-rich and because GC-rich DNA is more compact in structure, it therefore follows that animal and plant SATACs will differ in function. The Office Action further states that references have been made of record that support this premise.

It is respectfully submitted that while the references provided and made of record by Applicant teach the high GC content of plant satellite DNA, none of the references suggest that the GC content, nor compact structure of GC-rich DNA, impacts function. In fact, as discussed by Applicant responsive to the previous Office Action, the art cited in the previous Office Action, such as Biochemistry & Molecular Biology of Plants (Buchanan, Gruiseem, Jones, eds. 2000) specifically states that although sequence composition differs among species, the organization and arrangement of repetitive DNA (satellite DNA) in centromere regions is highly conserved (page 325, column 1, 1st paragraph). Additionally, as discussed in the previous response dated July 16, 2003, evidence in the literature cited by the Examiner, such as Avramova *et al.* (*Plant Physiol.* (2002) 129: 404-49) suggests strong similarity of structural elements of chromosomes of between plants and animals. Furthermore, as described in Bennett *et al.* ((1983) *J. Cell Sci.* 63:173-79, provided herewith), the density of metaphase chromosomes for mammalian cells and plant cells is within the same

range (*see*, for example, page 177, paragraph 2). The authors of Bennett *et al.* further state that there data confirms the conclusion of other researchers that "there is no consistent difference in the compaction of plant vs animal chromosomes" (page 177, paragraph 2).

Therefore, Applicant respectfully submits that the GC-rich nature of some plant DNAs does not evidence a predictable difference in function between plant and animal SATACs. In addition, the DECLARATION I submitted previously and DECLARATION II provided herewith demonstrate that by following the teachings of the specification, one of skill in the art can make a plant SATAC and can transfer SATACs into plant cells. The DECLARATIONS further evidence the applicability of the teachings of the instant application to a variety of species, including plants and animals.

7) The Office Action also alleges that Willard *et al.*, cited in the previous Office Action as a post-filing date reference, evidences the inoperability of the claimed subject matter years after the filing date, which in turn demonstrates lack of the full scope of enablement as of the effective filing date of the instant application.

Applicant respectfully submits that Willard *et al.* makes no such statements or implications that pertain to operability of the claimed subject matter. In contrast, Willard *et al.* specifically states that as of 2001, at least three research groups had successfully produced fully functional artificial chromosomes (*see* page 1309, column 1, seconds paragraph). Further, references cited by Willard *et al.*, *e.g.*, Harrington *et al.*((1997) *Nat. Genet.* 15: 345-355), demonstrate the transfection of alpha-satellite DNA to produce microchromosomes in human cells at a relatively high frequency (page 352, column 1, paragraph 1). Therefore, it is respectfully submitted that Willard *et al.* cannot be used as a post-filing date reference to show inoperability, when it clearly states the converse of that assertion. Other teachings of Willard *et al.*, such as mapping specific functions (spindle attachment, *e.g.*) within a

centromere, also have no bearing on operability of the instantly claimed methods of producing cells containing heterologous nucleic acid by introducing SATACs into cells. Furthermore, teachings of Willard *et al.* are irrelevant to the instant application, which teaches and describes transfer of SATACS.

8) The Office Action also refers to other references cited by the Examiner in the previous Office Action, reiterating the allegation that these references demonstrate the unpredictability of the art. (Telenius *et al.* ((1999) *Chrom. Res.* 7:3-7) and Shen *et al.* ((1999) *Curr. Biol.* 10:31-34). It is alleged that centromeres show species-specific behavior and thus it would be unpredictable that plant centromeres would be structurally and biochemically the same as animals.

In response, it is respectfully submitted that these references do not demonstrate species-specific behavior of centromeres. For example, Telenius *et al.* successfully maintains a mouse SATAC in mouse, bovine and human cells for over three months and additionally demonstrates maintenance of the SATAC in each of these species of cells in the absence of selection (paragraph spanning page 5, column 2 to page 6, column 1). Shen *et al.* demonstrates the stability of a 4.5 kb minichromosome in mouse, human and chicken cells (page 33, paragraph spanning columns 1-2). Thus, each reference evidences the success of artificial chromosome technology across species and can not be used as a post-effective filing date reference to demonstrate inoperability. Furthermore, as described in the application (and as demonstrated in the DECLARATION submitted herewith), particular differences among centromeres from different species does not impact upon SATAC formation.

9) Responsive to Applicant's argument filed responsive to the previous Office Action that there is evidence for chromosomal amplification events in plants, it is alleged that the argument is not persuasive because the references cited by Applicant in support thereof, U.S. Patent Nos. 6,355,860, and

6,100,092, are post-filing date references that cannot be used to support enablement as of the date of filing.

It is respectfully submitted that the aforementioned references were not cited to support enablement of the instantly claimed subject matter. Further subject matter therein regarding knowledge of the existence of amplifiable elements in plants predates issuances of such patents by many years. As discussed above, the specification is enabling for the generation of a SATAC, including a plant SATAC, and as the DECLARATION filed herewith (DECLARATION II) demonstrates, also demonstrates that these methods are operatives. The specification teaches the construction of SATACs, including plant SATACs, in great detail. Further, DECLARATION I submitted responsive to the previous Office Action and DECLARATION II submitted herewith demonstrate that by following the teachings of the application, SATACs can be introduced into cells, including cells of a different species, and plant SATACs can be generated and stably maintained in plant cells.

U.S. Patent Nos. 6,355,860, and 6,100,092, were cited in the previous response filed July 16, 2003, as evidence that plants do have amplifiable regions. **Knowledge of the existence of these amplifiable regions, which goes to the mechanism of SATAC formation, is not necessary for enablement of the instant methods** and predates issuance of those patents. As discussed above and as taught by the specification, for generation of a SATAC in any species, it is only necessary to introduce a piece of DNA, grow the cell,look for cells that contain satellite artificial chromosomes and select such cells. A satellite artificial chromosome can be isolated therefrom. The US Patent Nos. 6,355,860, and 6,100,092, were cited only to show that there is no evidence of record that suggests that the aforementioned steps are applicable only to generate SATACs of animal species. The cited patents would suggest that the amplification event leading to generation of a SATAC can occur in plants. The patents are cited to support the contention that such amplifiable regions exist (not to teach how to

make and use any aspect of the claimed methods). The operability of the instant methods is further demonstrated by DECLARATION II, submitted herewith, that describes the generation of a plant SATAC and its stable maintenance in plant cells.

10) The Office Action further alleges that because the examples in the above cited references are directed to animals and not plants, they therefore support the unpredictability of the art with respect to plants. There is no basis in the record for such statement or conclusion.

It is respectfully submitted that references that lack teachings directed to plant artificial chromosomes provide no evidence as to the predictability of their generation and maintenance in cells. None of the references cited by the Examiner offers any evidence that plant artificial chromosomes would be structurally or functionally different from mammalian artificial chromosomes. There is no teaching within these references that would suggest inoperability of plant SATACs or of transferring SATACs to plants. The absence of the Applicant's claimed subject matter in the literature does not *a priori* support an enablement rejection. Such rejections may only be made where individuals of skill in the art state that a particular invention is not possible. In re Wright, 999 F.2d 1557, 1562, 27 USPQ2d 1510, 1513-14 (Fed. Cir. 1993). Since none of the references make such assertions regarding methods of producing cells with plant SATACs, use of these post-filing references to evidence non-enablement is improper.

Furthermore, even if, *arguendo*, post-filing dated references somehow suggested the claimed subject matter was inoperable, the DECLARATION I submitted previously and DECLARATION II submitted herewith and discussed in detail below evidence otherwise. The DECLARATIONS demonstrate that by following the teachings of the specification, the methods operate as claimed for plant SATACs and for the transfer of SATACs into plant cells. The DECLARATIONS show that, by following the teachings of the instant

application, plant SATACs can be generated and maintained in plant cells and SATACs can be introduced into plant cells. In addition, DECLARATION I demonstrates that SATACs of one species can be introduced and maintained in cells of a different species.

11) With regard to the DECLARATION (DECLARATION I) filed responsive to the previous Office Action, it is alleged that the results provided in DECLARATION I are unpersuasive because DECLARATION I states that the microcells for introduction into tobacco cells were prepared from murine cells containing a SATAC as described in U.S. Patent No. 6,077,697, which is a post-filing date reference. It is further alleged that DECLARATION I is "lacking any teaching of a plant SATAC, a plant SATAC in a plant cell, a plant SATAC in a plant, or a plant SATAC in an animal cell."

The instant application is a continuation-in-part of the application corresponding to issued U.S. Patent No. 6,077,697. The application, U.S. Application No. 08/682,080, is incorporated in its entirety by reference herein and is explicitly included in the application. Further, the entire subject matter related to the preparation of microcells from murine cells (EC3/7C5) containing a SATAC was first described in U.S. Application No. 08/629,822, filed April 10, 1996 and now abandoned, of which U.S. Application No. 08/682,080, and the instant application are continuations-in-part. U.S. Application No. 08/629,822, is also incorporated in its entirety by reference herein. The specification further describes these methods in great detail (*see, e.g.*, page 51, lines 15-26; page 70, line 14 to page 72, line 14; Example 3 beginning at page 81; page 136, line 12 to page 137, line 2). Therefore, it is respectfully submitted that while the DECLARATION refers to the publication of the disclosure that is incorporated by reference in the instant application, the methods used in DECLARATION I follow the teachings of the specification as described an incorporated by reference therein as of its earliest priority date (April 10, 1996).

Further, in addition to microcell-mediated methods that were known to those of skill in the art and described and incorporated by reference in the specification as of its earliest filing date, the DECLARATION I demonstrates other methods for introducing and identifying animal SATACs in plant cells. DECLARATION I describes the introduction of a SATAC into tobacco and Arabidopsis cells by a variety of methods. In addition to microcell-mediated fusion of mouse SATACs with tobacco protoplasts and Arabidopsis protoplasts, the DECLARATION I also describes the lipid-mediated transfer of isolated SATACs (*i.e.*, not in microcells) into rice cells. It is therefore respectfully submitted that the Examiner has provided no reason why the DECLARATION fails to demonstrate operability of the claimed methods in which a SATAC of one species (mammal) can be transferred and stably maintained in cells of another apecies (plant). Contrary to the Examiner's assertion, DECLARATION I clearly demonstrates that using the procedures as taught in the above-captioned application, satellite artificial chromosomes can be delivered to plant cells. The results demonstrate that delivery of animal satellite artificial chromosomes to plant cells and their stable maintenance and identification in plant cells can be achieved by methods well known to those of skill in the art and described and incorporated by reference in the specification, including microcell-mediated fusion and lipid-mediated transfection.

The Examiner alleges that DECLARATION I fails to teach a plant SATAC, a plant SATAC in a plant cell, a plant SATAC in a plant or a plant SATAC in an animal cell. First, it is respectfully submitted that the experiments and described in the DECLARATION follow the teachings of the specification, and the specification teaches the elements of the subject matter as claimed. Moreover, as discussed in great detail above, the specification teaches in great detail the components of a SATAC, methods for their generation, methods to identify the SATACs, detailed characterization of the SATACs, introduction of SATACs into cells, the generation of transgenic animals and plants, and the applicability of

the teachings to SATACs and to cells of any species, including animals and plants. As discussed above, Applicant is not required to exemplify every embodiment of the claimed subject matter. DECLARATION I demonstrates that by following the teachings of the specification, SATACs can be introduced into plant cells, and the SATACs are identified by, *e.g.*, fluorescence *in situ* hybridization (FISH). Thus, DECLARATION I demonstrates the operability of methods of introducing SATACs into cells, including SATACs of one species into cells of another species, and identifying the SATACs. Further, as discussed above, the specification provides additional examples characterizing the SATACs and demonstrating the introduction of, *e.g.*, animal SATACs into animal cells. As discussed in the previous response filed July 16, 2003, there is no evidence of record, nor has the Examiner provided any, that suggests that such events are unique to animal cells, nor is there any reason to believe such. On the contrary, the specification teaches that the events and the methods based thereon are applicable to cells from any eukaryotic species.

Regardless, as discussed below and above, DECLARATION II submitted herewith demonstrates that by following the teachings of the specification, a plant SATAC can be generated and stably maintained in a plant cell. Therefore, it is respectfully submitted that the specification, in light of the knowledge of those of skill in the art, teaches one of skill in the art how to generate a transgenic plant by generating a SATAC of any species and further introducing a SATAC of any species into a protoplast.

DECLARATION II

Notwithstanding the above arguments, to evidence that the methods as claimed operate as claimed, attached is a DECLARATION (DECLARATION II) under 37 C.F.R. §1.132 of Steven F. Fabijanski. DECLARATION II shows that by following the teachings of the application as of its earliest filing date, plant SATACs can be generated and maintained in plant cells.

Also, although DECLARATION II is submitted to rebut the Examiner's assertions of inoperativeness, it also further evidences enablement. It is noted that the level of skill in the biotechnical arts is recognized to be high (see, *e.g.*, Ex parte Forman, 230 USPQ 546 (Bd. Pat. App. & Int'f 1986). Further, methods for performing the various steps of the claimed methods, such as preparation of microcells, microcell-mediated fusion, lipid-mediated transfection and techniques for the detection of specific DNA sequences (*e.g.*, satellite artificial chromosomes) in recipient cells, constructing heterologous DNAs and introducing DNA, including large DNA such as artificial chromosomes, into a cell were known to the skilled artisan at the time of filing.

Dr. Fabijanski is not an inventor of this application. In performing or directing the experiments in DECLARATION II, he followed the teachings in the application. Since those of skill in this art typically have advanced degrees, Dr. Fabijanski, who has an Ph.D. degree, is representative of a person of skill in this art with respect to performing experiments in accord with a disclosed protocol.

The DECLARATION II demonstrates that, using the methods as taught in the above-captioned application, plant SATACs can be generated and maintained in plant cells.

Specifically, the DECLARATION II of Dr. Fabijanski demonstrates that by following the teachings in the application, plant SATACs can be generated by i) introducing a DNA fragment with a selectable marker into a plant cell; ii) growing the cell under selective conditions to produce plant cells that have incorporated the DNA into their genomic DNA such that a plant SATAC is produced; and iii) selecting a cell that contains a plant SATAC. The plant SATAC contains a plant centromere, as well as amplified pericentric DNA and the introduced heterologous DNA. The plant SATAC can be detected in cells and the cells can be maintained in culture for over 6 months.

Heterologous DNA containing DNA with homology to the pericentric region of plant chromosomes, a selectable marker and a β-glucuronidase (GUS)

reporter gene was introduced into tobacco cells. Cell lines (calli) that expressed the GUS reporter were selected. Fluorescence *in situ* hybridization (FISH) was used to demonstrate the formation of a sausage chromosome and a resulting plant SATAC. The plant cell line with a plant SATAC was maintained for over 6 months in culture.

The results of these analyses demonstrate that the methods described in the above-referenced application can be used to generate, identify and maintain plant SATACs in plant cells. The previous DECLARATION (DECLARATION I) demonstrated that by following the teachings of the application, it is possible to introduce SATACs into plant cells, and the SATACs may further be derived from another species, such as mouse. Therefore, the DECLARATIONS provide further evidence that the disclosure in the above-captioned application enables methods for introducing SATACs into plant cells and for generating plant SATACs that can be used with the methods in the instant application. The DECLARATIONS demonstrate element-for-element and step-for-step that, by following the teaching in the application, one can: i) introduce a DNA fragment with a selectable marker into a plant cell; ii) grow the cell under selective conditions to produce plant cells that have incorporated the DNA into their genomic DNA such that a plant SATAC is produced; and iii) select a cell that contains a plant SATAC. DECLARATION I further demonstrates that by following the teachings in the application, a SATAC can be introduced into a plant cell. Accordingly, Applicant respectfully submits that the claims are commensurate in scope with the Applicant's discovery and its disclosure within the above-captioned application.

Applicant also wishes to point out that the DECLARATIONS I and II follow the teachings as disclosed in the instant application and in the parent applications, U.S. Patent Nos. 6,025,155 and 6,077,697, and U.S. Application Serial Nos. 08/835,682 and 08/629,822. The DECLARATIONS demonstrate the use of DNA targeted to the pericentric region of the chromosome for the

generation of a plant SATAC, and the introduction of SATACs into plant cells, both of which are taught by the instant application and the parent applications.

* * *

In view of the above amendments and remarks, reconsideration and allowance of the application are respectfully requested.

Respectfully submitted,
FISH & RICHARDSON P.C.

By: _____
Stephanie Seidman
Registration No. 33,779

Attorney Docket No. 24601-402E (Current: 17084-004006)

Address all correspondence to:
Stephanie Seidman
FISH & RICHARDSON P.C.
12390 El Camino Real
San Diego, CA 92130-2081
Telephone: 858 678-5070
Facsimile: 202 626-7796
email: seidman@fr.com

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: HADLACZKY *et al.*

Serial No.: 09/724,726
Filed: November 28, 2000
Customer No.: 20985
Confirmation No: 7776

For: *ARTIFICIAL CHROMOSOMES, USES THEREOF AND METHODS FOR PREPARING ARTIFICIAL CHROMOSOMES*

Art Unit: 1638

Examiner: Helmer, G.L.

CERTIFICATE OF MAILING BY "EXPRESS MAIL"
"Express Mail" Mailing Label Number
EV 399293946 US
Date of Deposit April 22, 2004

I hereby certify that this paper and the attached papers are being deposited with the United States Postal "Express Mail Post Office to Addressee" Service under 37 C.F.R. §1.10 on the date indicated above and addressed to:

Commissioner for Patents
U.S. Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450

04/22/04 Stephanie Seidman

ATTACHMENTS TO RESPONSE

(1) Executed Declaration under 37 C.F.R. §1.132 of Dr. Steven Fabijanski.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: HADLACZKY *et al.*

Serial No.: 09/724,726 Group Art Unit: 1638

Filed: November 28, 2000 Examiner: Helmer, G.L.

For: ARTIFICIAL CHROMOSOMES, USES THEREOF AND METHODS FOR PREPARING ARTIFICIAL CHROMOSOMES

DECLARATION PURSUANT TO 37 C.F.R. §1.132

Sir:

I, Steven F. Fabijanski, declare as follows:

1) I am familiar with the subject matter of the above-captioned application, which was filed on November 28, 2000.

2) I received a Bachelor's degree in Biology from the University of Miami (Florida) in 1977. I received a Ph.D. degree in Cellular and Molecular Biology from the University of Southern California in 1981. I have held post-doctoral positions at the University of Ottawa in Ottawa, Ontario, Canada and the University of Southern California in Los Angeles, California from 1982 to 1985. From 1986 to 1991, I held the position of Research Director at Paladin Hybrids, Inc.

3) I have over 20 years of experience in the areas of plant molecular biology, plant gene expression, plant tissue and cell culture and development of techniques to produce genetically modified plants and plant artificial chromosomes. I have authored or co-authored over 20 publications and I am an inventor of 15 US and foreign patents.

4) I am currently Research Director at Agrisoma Biosciences Inc., located in Saskatoon, Saskatchewan, Canada. Chromos Molecular Systems, Inc., located at 8081 Lougheed Highway, Burnaby, B.C., Canada V5A 1W9, is an owner of Agrisoma Biosciences, Inc. I have held this position since 2001. I am also President of FAAR Biotechnology Group Inc., located at Suite 323, 5929L Jeanne D'arc Boulevard, Orleans, Ontario, Canada K1C 7K2. I have held this position since 1992.

5) In my capacity as researcher, myself, persons under my direction and other research groups: the Scottish Crop Research Institute in Scotland; the Danforth Plant Science Center in St. Louis, Missouri; the Hungarian Biological Research Center in Hungary; and Applicant's research group at the Plant Biotechnology Institute in Saskatoon, Saskatchewan, Canada; have studied generation of plant artificial chromosomes.

6) Using methods and materials described in the above-referenced application and standard methods as described herein, myself and other scientists involved in these projects have demonstrated that plant artificial chromosomes can be generated by introduction of heterologous DNA into plant cells and growth under selective conditions to produce cells containing plant satellite artificial chromosomes.

As exemplified by the results shown below, we have demonstrated element-for-element and step-for-step that, by following the teachings in the application, plant artificial chromosomes can be generated by i) introducing a DNA fragment with a selectable marker into a plant cell; ii) growing the cell under selective conditions to produce plant cells that have incorporated the DNA into their genomic DNA such that a plant satellite artificial chromosome is produced; and iii) selecting a cell that contains a plant satellite artificial chromosome.

MATERIALS AND METHODS

Generation of Plant Artificial Chromosomes (Plant SATACs)

1. Construction of heterologous DNAs

Vector pAgIIa, containing two selectable markers and a sequence with homology to the pericentric DNA, was constructed using standard techniques of molecular biology. A hygromycin phosphotransferase (HPT) gene under the control of the 35S promoter (see, for example, Blochinger *et al. Mol. Cell. Biol.* 4:2929-2931) was incorporated into the vector for selection. A 334 base pair sequence with homology to tobacco pericentric sequences (Genbank Accession No.

Y08422, submitted 1996; see also Genbank Accession Nos. X76056 and D76443 submitted 1993 and 1995, respectively) was constructed, containing the central AT-rich region of a tobacco rDNA intergenic spacer capable of amplification (Borisjuk *et al.* (1997) *Plant Mol. Biol.* 35:655-660). Vector pAGIIa also contains a visible marker, constructed by placing a β-glucuronidase (GUS) gene under the control of the nos promoter (Novel *et al.* (1973) *Mol. Gen. Genet.* 120:319-335; Jefferson *et al.* (1986) *Proc. Natl. Acad. Sci. USA* 83:8447-8451; US Patent No. 5,268,463; commercially available from Clontech Laboratories, Palo Alto, CA) and a detection marker containing a 234 base pair mouse major satellite DNA sequence derived from pSAT-1 (Wong *et al.* (1988) *Nucleic Acid Research*, 16(24):11645-11661. The vector also contains a second selectable marker constructed from a phosphinothricin acetyl transferase (PAT) gene under the control of the 35S promoter, (see for example, White *et al.* (1990) *Nuc. Acids Res.* 18:1062; Spencer *et al.* (1990) *Theor. Appl. Genet.* 79:625-631; Vickers *et al.* (1996) *Plant Mol. Biol. Reporter* 14:363-368; and Thompson *et al.* (1987) *EMBO J.* 6:2519-2523).

A targeting DNA was constructed with homology to pericentric DNA sequences. The targeting DNA contains a 1.7 Kb portion of the 26S rDNA coding region (Genbank accession X52320). The targeting DNA was cloned into the vector pBluescript (Stratagene, La Jolla, CA).

2. Introduction of DNAs into plant cells and selection

Vector DNA and targeting DNA were introduced into tobacco cells using PEG mediated transfection. Briefly, tobacco protoplasts were isolated from established sterile tobacco plant cultures by immersion of sterile tissue in enzyme solution containing 1.2% Cellulase 'Onozuka' R-10 and 0.4% Macerozyme R-10. The protoplasts were purified by pouring through a 100 μm nylon mesh sieve, overlaid with washing solution and centrifuged at 80 x g for 10 min. Protoplasts were then resuspended at a density of 1 $x10^6$ protoplasts/ml and stored at 4°C for 1 to 2 hours prior to DNA uptake.

The vector and targeting DNAs were sterilized with chloroform and 70% ethanol before use. A protoplast suspension was mixed with vector and targeting DNA at a ratio of 1:10, followed immediately by slow addition of a polyethylene glycol (PEG) solution. As controls, salmon sperm or calf thymus DNA was added instead of the targeting DNA. The mixture was incubated at 22°C for 10-15 min, with gentle shaking. The protoplasts were resuspended and cultured at 22°C in the dark. When microcalli developed, the protoplasts were embedded in 0.6% agarose. Selection on protoplast cultures was carried out by adding hygromycin to the medium at a final concentration of 20 mg/l, 14 to 21 days after transfection.

Calli that grew on selection were cultured under selective conditions for a period of 3 - 6 months, with frequent subculturing. Standard molecular biology techniques were used to verify the presence of the vector DNA.

3. Identification of amplified DNAs

GUS-expressing calli produced using vector DNA and either the targeting DNA or control DNA were subjected to two-color Fluorescent In Situ Hybridization (FISH) using two probes. The first probe was tagged with rhodamine (red fluorescence) and recognized pericentric DNA (18S rDNA) sequences endogenous to tobacco cells. The second probe recognized the detection marker (mouse major satellite sequence) in the pAgIIa vector used for transfection and was visualized with a fluorescein isothiocyanate (FITC) tag (blue-green fluorescence).

To obtain spreads of metaphase chromosomes, cells were subjected to either a single blocking protocol (colchicine treatment), or double blocking protocol (for example, treating plant cells with 5 mg/L aphidicolin for 24 hours and then 1.54 mg/ml Propyzamide for 4 hours). The blocked cells were recovered and chromosome spreads prepared and subjected to two-color FISH. Red and blue-green fluorescence was monitored to identify amplification. In general, 8-10 chromosome spreads were screened per sample. Further fluorescent image analysis was performed in a subset of the samples to overlay the probe signals and further detail chromosome structure.

RESULTS

The results demonstrate that plant artificial chromosomes (plant SATACs) can be generated and maintained in plant cells.

Following introduction of the heterologous DNAs into tobacco cells, cells were selected on hygromycin. More than 400 calli were obtained. A portion of the calli were analyzed for expression of the GUS reporter gene. A total of 31 independent GUS-expressing calli obtained using the targeting DNA or control DNAs were selected for further analysis.

The calli were subjected to two-color FISH using the probes for endogenous pericentric (18S rDNA) sequences and for the detection marker in the vector. The endogenous pericentric (rDNA) loci on tobacco chromosomes stained red, and regions of the chromosome where the vector DNA inserted stained blue-green. Where amplification of the vector DNA had occurred, medium to high blue-green signal was observed. A low signal blue-green signal indicated a lack of large scale amplification of the vector sequence. Twenty-four calli produced with the vector and targeting DNA were analyzed for blue-green signal level: 5 were categorized as a medium level signal, 1 as high and 1 as medium high. The remaining 17 samples produced a low blue-green signal. The control calli, produced with vector DNA and either salmon sperm or calf thymus control DNAs, all produced low levels of blue-green signal. The probe for 18S rDNA recognizes the native pericentric sequences (rDNA) found in the tobacco chromosomes and thus should recognize all samples equivalently. This probe provided a red signal of high intensity in all of the analyzed samples.

Thus, in 7 out of 24 analyzed calli produced with targeting DNA homologous to pericentric sequences, large scale amplification of the vector sequences was observed at the chromosome level. No such amplification was observed using salmon sperm or calf thymus DNAs, indicating that targeting DNA without known homology to pericentric DNA is less efficient for stimulating large scale amplification.

Overlap analysis of the probes was used to further determine the fate of targeting and vector DNAs. Overlap of blue-green and red signals indicated that homologous recombination of the targeting DNA and vector DNA had occurred. Additionally, areas where significant levels of both blue-green and red signals were observed, demonstrated large scale amplification of pericentric regions.

One of the 7 callus lines with a medium-high vector signal was analyzed further. This line was shown to contain a chromosome that exhibited large scale "sausage" amplification and a breakage product representing a plant artificial chromosome (plant SATAC). Comparison of the rhodamine and FITC signals demonstrated that the vector was inserted and amplified in two of the eight visible pericentric (rDNA) loci. The first locus was identified as a "sausage" amplification by the tandemly duplicated nature of the vector and pericentric DNA signals. This locus represented a formerly dicentric chromosome and/or a sausage chromosome. The second locus identified a breakage product, indicating the creation of a plant SATAC by breakage of a formerly dicentric chromosome. The plant SATAC contained vector sequence integrated into plant pericentric satellite DNA.

The SATAC was clearly visible in chromosome spreads as a small independent chromosome entity containing both amplified vector DNA and pericentric DNA. Image analysis was used to overlay both pericentric DNA and vector DNA signals. In high resolution images, the presence of both amplified vector (blue-green signal) and pericentric heterochromatic DNA (red signal) was repeatedly observed. Metaphase images of the SATAC demonstrated the presence of small chromosome arms and a constriction representing the centromere region. Because amplified DNA sequences were detected with the probe specific for pericentric sequences (18S rDNA), it represented newly amplified DNA. This signal can not be attributed to the introduced targeting DNA used, because the targeting DNA contained only 26S rDNA sequences.

Thus, this callus line demonstrated the production of two amplified regions as a result of insertion of vector DNA into the pericentric DNA. The analysis

indicated targeting of the vector DNA to pericentric DNA and evidence for large scale amplification, including "sausage" amplification. A SATAC, produced from a breakage product, was identified that contained amplified vector DNA as well as heterochromatic DNA. The callus line containing the plant SATAC was stably maintained in culture for over 6 months.

CONCLUSION

The above experiments demonstrate that a plant artificial chromosome (plant SATAC) can be generated by introducing heterologous DNA into a plant cell, growing cells under selective conditions to produce cells that have incorporated the DNA such that a plant satellite artificial chromosome is produced and selecting a cell that contains a plant SATAC. The plant SATAC contains a plant centromere, as well as amplified pericentric DNA and the introduced heterologous DNA. The plant SATAC can be detected in cells and the cells can be maintained in culture for over 6 months.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent resulting therefrom.

Jan 19 2004
Date

Steven F. Fabijanski